United States Environmental Protection Agency | Office of Research and Development Washington DC 20460 | EPA/600/BP-92/001b June 1994 External Review Draft

EPA Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds Review Draft (Do Not Cite or Quote)

Volume II of III

Notice

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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Next Stages in the Reassessment Process

As described previously, public briefings will be held during the first week of the public comment period to be followed by formal public hearings in December 1994. After the close of the public comment period, the Agency's Science Advisory Board (SAB) will review the draft documents in public session (early 1995). Following SAB review, the draft documents will be revised, comments and revisions will be incorporated, and final documents will be issued.

8/30/94
Date

Acting Carl R. Gerber
Assistant Administrator
for Research and Development

Billing Code: 6560-50-P

ecosystems from exposure to dioxins. Research efforts are focused on the study of organisms in aquatic food webs to identify the effects of dioxin exposure that are likely to result in significant population impacts. A report titled, *Interim Report on Data and Methods for the Assessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Risks to Aquatic Organisms and Associated Wildlife* (EPA/600/R-93/055), was published in April 1993. This report will serve as a background document for assessing dioxin-related ecological risks. Ultimately, these data will support the development of aquatic life criteria which will aid in the implementation of the Clean Water Act.

As mentioned previously, completion of the health assessment and exposure documents involves three phases: Phase 1 involved drafting state-of-the-science chapters and a dose-response model for the health assessment document, expanding the exposure document to address dioxin related compounds, and conducting peer-review workshops by panels of experts. This phase has been completed.

Phase 2, preparation of the risk characterization, began during the September 1992 workshops with discussions by the peer-review panels and formulation of points to be carried forward into the risk characterization. Following the September 1993 workshop, this work was completed and was incorporated as Chapter 9 (Volume III) of the draft health assessment document. This phase has been completed.

Phase 3 is currently underway. It includes making External Review Drafts of both the health assessment document and the exposure document available for public review and comment.

scientific experts from outside the Agency reviewed the draft documents and provided valuable comments. It also should be noted that outside scientists have been heavily involved throughout the developmental process of writing and reviewing these draft documents. With this notice, the External Review Drafts of both draft documents are being released for a 120-day public review and comment period.

Current Stage of the Scientific Reassessment of Dioxin

The scientific reassessment of dioxin consists of five activities:

1. Update and revision of the health assessment document for dioxin.
2. Laboratory research in support of the dose-response model.
3. Development of a biologically based dose-response model for dioxin.
4. Update and revision of the dioxin exposure assessment document.
5. Research to characterize ecological risks in aquatic ecosystems.

The first four activities have resulted in two draft documents (the health assessment document and exposure document) for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. These companion documents, which form the basis for the Agency's reassessment of dioxin, have been used in the development of the risk characterization chapter that follows the health assessment (Chapter 9, Volume III). The process for developing these documents consisted of three phases which are outlined in later paragraphs.

The fifth activity, which is in progress at EPA's Environmental Research Laboratory in Duluth, Minnesota, involves characterizing ecological risks in aquatic

to hear and receive public comments and reviews of the proposed plans, and to receive any current, scientifically relevant information.

In the Fall of 1992, the Agency convened two peer-review workshops to review draft documents related to EPA's scientific reassessment of the health effects of dioxin. The first workshop was held September 10 and 11, 1992, to review a draft exposure assessment titled, *Estimating Exposures to Dioxin-Like Compounds*. The second workshop was held September 22-25, 1992, to review eight chapters of a future draft *Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds*. Peer-reviewers were also asked to identify issues to be incorporated into the risk characterization, which was under development.

In the Fall of 1993, a third peer-review workshop was held on September 7 and 8, to review a draft of the revised and expanded Epidemiology and Human Data Chapter, which also would be part of the future health assessment document. The revised chapter provided an evaluation of the scientific quality and strength of the epidemiology data in the evaluation of toxic health effects, both cancer and noncancer, from exposure to dioxin, with an emphasis on the specific congener, 2,3,7,8-TCDD.

Prior to each workshop, the draft documents or chapters were made available in keeping with the Agency's continuing commitment to conduct the reassessment of dioxin in an open and participatory manner, to keep the public informed of its progress, and to encourage public participation in the document development process. The public also was invited to attend the workshops, to present oral comments, and/or to submit written comments. At each workshop, a panel of

adverse health effects of dioxin in people, of the pathways to human exposure, and of the toxic effects of dioxin to the environment. The reassessment is part of the Agency's goals to improve the research and science base and to incorporate improved research and science into EPA decisions.

History

In 1985 and 1988, the Agency prepared assessments of the human health risks from environmental exposures to dioxin. Also, in 1988, a draft exposure document was prepared that presented procedures for conducting site-specific exposure assessments to dioxin-like compounds. These assessments were reviewed by the Agency's Science Advisory Board (SAB). At the time of the 1988 assessments, there was general agreement within the scientific community that there could be a substantial improvement over the existing approach to analyzing dose response, but there was no consensus as to a more biologically defensible methodology. The Agency was asked to explore the development of such a method. The Agency's reassessment activities are in response to this request.

Stages in the Reassessment Process That Have Been Completed

The EPA had endeavored to make each phase of the reassessment of dioxin an open and participatory effort. On November 15, 1991, and April 28, 1992, public meetings were held to discuss the Agency's plans and activities for the reassessment,

For the exposure assessment document, send comments to: Dioxin Exposure Assessment Comments, Technical Information Staff (8601), Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460.

FOR FURTHER INFORMATION, CONTACT:

For questions on the overall reassessment of dioxin or technical questions on the health assessment document: William Farland, Office of Health and Environmental Assessment (8601), Office of Research and Development, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460; telephone (202) 260-7315; fax (202) 260-0393.

For technical questions on the exposure assessment: John Schaum, Exposure Assessment Group (8603), Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460; telephone (202) 260-8909; fax (202) 260-1722.

SUPPLEMENTARY INFORMATION:

The Scientific Reassessment of Dioxin

In April 1991, EPA announced that it would conduct a scientific reassessment of the health risks of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and chemically similar compounds collectively known as dioxin. The EPA has undertaken this task in response to emerging scientific knowledge of the biological, human health, and environmental effects of dioxin. Significant advances have occurred in the scientific understanding of mechanisms of dioxin toxicity, of the carcinogenic and other

Agency, 26 W. Martin Luther King Drive, Cincinnati, OH 45268; telephone (513) 569-7562; fax (513) 569-7566. Please provide your name, mailing address, document title, and EPA number.

Please note that the two summary volumes also will be made available as WordPerfect 5.1 files on 3½" PC-DOS formatted disks. Please request by document title and EPA number:

Risk Characterization Chapter (Vol. III-Health), EPA/600/BP-92/001ca (disk)

Executive Summary Chapter (Vol. I-Exposure), EPA/600/6-88/005Caa (disk)

The draft documents will be provided for inspection at the ORD Public Information Shelf, EPA Headquarters Library, 401 M Street, S.W., Washington, DC 20460, between the hours of 10:00 a.m. and 2:00 p.m., Monday through Friday, except for Federal holidays, and at all of the EPA Regional and Laboratory libraries.

Submitting Comments

All comments must be in writing. Commenters should submit three copies of each comment, and if commenting on both documents—the health assessment document and the exposure assessment—submit separate comments rather than combined submissions.

For the health assessment document, send comments to: Dioxin Health Assessment Comments, Technical Information Staff (8601), Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460.

ADDRESSES:

Requesting Documents

Due to the large size of both draft documents (each is over 1,000 pages in length), the documents will be available as follows:

Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds, EPA/600/BP-92/001a, 001b, 001c. (Note: The full document is 3 volumes and approx. 1,100 pages.)

OR

Risk Characterization Chapter, EPA/600/BP-92/001c. (Note: This third volume of the 3-volume set integrates health and exposure information on dioxin and related compounds; approx. 100 pages.)

AND/OR

Estimating Exposure to Dioxin-Like Compounds, EPA/600/6-88/005Ca, Cb, Cc. (Note: The full document is 3 volumes and approx. 1,300 pages.)

OR

Executive Summary Chapter of the Exposure Document, EPA/600/6-88/005Ca. (Note: This first volume of the 3-volume set summarizes the exposure information; approx. 100 pages.)

To obtain a paper copy of these draft documents, interested parties should contact the ORD Publications Center, CERI-FRN, U.S. Environmental Protection

Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, dioxin)

AGENCY: U.S. Environmental Protection Agency (EPA)

ACTION: As part of the Agency's reassessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD; hereinafter referred to as simply dioxin), two External Review Draft documents are being made available for a 120-day public review and comment period.

SUMMARY: This notice announces the availability of two External Review Draft documents for public review and comment:

1. *Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds* (EPA/600/BP-92/001a-c)
2. *Estimating Exposure to Dioxin-Like Compounds* (EPA/600/6-88/005Ca-c)

During the public comment period, public comment meetings will be convened to take formal comments on the draft documents. These meetings are being planned for the first two weeks of December at five locations: Washington, DC; New York, NY/New Jersey; Chicago, IL; Dallas, TX; and San Francisco, CA. Detailed information will be provided in a future *Federal Register* notice.

The draft documents also will be reviewed at a Science Advisory Board meeting to be held after the public comment period has ended, early next year. Information about this meeting will be published in a future *Federal Register* notice.

DATES: The draft documents will be made available on September 13, 1994. Comments must be postmarked by January 13, 1994.

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EPA/600/BP-92/001b
June 1994
External Review Draft

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Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

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This document is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**Health Assessment Document for
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)
and Related Compounds**

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTH	Adrenocorticotrophic hormone
Ah receptor	Aryl hydrocarbon receptor
AHH	Aryl hydrocarbon hydroxylase
ALA	Aminolevulinic acid
ALT	L-alanine aminotransferase
AOR	Adjusted odds ratio
APC	Antigen-presenting cells
AST	L-aspartate aminotransferase
ATPase	Adenosine triphosphatase
BDD	Brominated dibenzo-*p*-dioxin
BDF	Brominated dibenzofuran
BCF	Bioconcentration factor
BGG	Bovine gamma globulin
bHLH	Basic helix-loop-helix
bw	Body weight
cAMP	Cyclic 3,5-adenosine monophosphate
CDC	Centers for Disease Control and Prevention
CDD	Chlorinated dibenzo-*p*-dioxin
CDF	Chlorinated dibenzofuran
cDNA	Complementary DNA
cl	Confidence level
CMI	Cornell Medical Index
CNS	Central nervous system
CSM	Cerebrospinal malformation
CTL	Cytotoxic T lymphocyte
DCDD	2,7-Dichlorodibenzo-*p*-dioxin

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

DEN	Diethylnitrosamine
DHT	5α-Dihydrotestosterone
DIS	Diagnostic Interview Schedule
DMBA	Dimethylbenzanthracene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DRE	Dioxin-responsive enhancers
DTH	Delayed-type hypersensitivity
EC_{50}	Concentration effective for 50% of organisms tested
EC_{100}	Concentration effective for 100% of organisms tested
ED_{50}	Dose effective for 50% of recipients
ECOD	7-Ethoxycoumarin-O-deethylase
EEG	Electroencephalogram
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
EROD	7-Ethoxyresorufin-O-deethylase
EOF	Enzyme altered foci
EOI	Exposure opportunity index
FEV	Forced expiratory volume
FIQ	Full-scale IQ
FSH	Follicle-stimulating hormone
FTI	Free thyroxine index
FVC	Forced vital capacity
GC-ECD	Gas chromatograph-electron capture detection
GC/MS	Gas chromatograph/mass spectrometer

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

GGT	Gamma glutamyl transpeptidase
GnRH	Gonadotropin-releasing hormone
GST	Glutathione-S-transferase
GVH	Graft versus host
HAH	Halogenated aromatic hydrocarbons
HCB	Hexachlorobenzene
HCDD	Hexachlorodibenzo-*p*-dioxin
HDL	High density lipoprotein
HLH	Helix-loop-helix
HPAH	Halogenated polycyclic aromatic hydrocarbon
HpCDD	Heptachlorinated dibenzo-*p*-dioxin
HpCDF	Heptachlorinated dibenzofuran
HPLC	High performance liquid chromatography
HRB	Halstead-Reitan Battery
HRGC/HRMS	High resolution gas chromatography/high resolution mass spectrometry
HTL	Human tonsillar lymphocytes
HxBB	Hexabrom-biphenyl
HxCB	Hexachlorobiphenyl
HxCDD	Hexachlorinated dibenzo-*p*-dioxin
HxCDF	Hexachlorinated dibenzofuran
ICD-9	International Classification of Diseases 9
ID_{50}	Dose infective to 50% of recipients
I-TEF	International TCDD-toxic-equivalency
KVK	Kemisk Vaerk Køge
LADD	Lifetime average daily dose
LD_{50}	Dose lethal to 50% of recipients (and all other subscripter dose levels)

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

LDH	L-lactate dehydrogenase
LH	Luteinizing hormone
LDL	Low density liproprotein
LMS	Linearized multistage
LPL	Lipoprotein lipase activity
LOAEL	Lowest-observable-adverse-effect level
LOEL	Lowest-observed-effect level
LPS	Lipopolysaccharide
MACDP	Metropolitan Atlanta Congenital Defects Program
3-MC	3-Methylcholanthrene
MCDF	6-Methyl-1,3,8-trichlorodibenzofuran
MCF-7	(breast cancer cell)
MCMI	Millon Clinical Multiaxial Inventory
MCPA	(4-Chloro-2-methylphenoxy)acetic acid
MCPB	2-Methyl-4-chlorophenoxybutyric acid
MCPP	2-(4-Chloro-2-methylphenoxy)-propanoic acid
MFO	Mixed function oxidase
MMPI	Minnesota Multiphase Personality Inventory
MLE	Maximum likelihood estimate
mRNA	Messenger RNA
MNNG	*N*-methyl-*N*-nitrosoguanidine
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NaTCP	Sodium 2,4,5-trichlorophenate
NHL	Non-Hodgkin's lymphoma
NIEHS	National Institute of Environmental Health Sciences

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

NIOSH	National Institute for Occupational Safety and Health
NK	Natural killer
NOAEL	No-observable-adverse-effect level
NOEL	No-observed-effect level
NTP	National Toxicology Program
OCDD	Octachlorodibenzo-*p*-dioxin
OCDF	Octachlorodibenzofuran
OR	Odds ratio
OVX	Ovariectomized
PAA	Phenoxyacetic acid
PAH	Polyaromatic hydrocarbon
PBA	Phenoxybutyric acid
PBB	Polybrominated biphenyl
PBF	Percent body fat
PBL	Peripheral blood lymphocytes
PB-PK	Physiologically based pharmacokinetic
PCB	Polychlorinated biphenyl
PCBA	Phenoxybutyric acid
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PCP	Pentachlorophenol
PCPA	Parachlorophenoxyacetic acid
PCQ	Quaterphenyl
PCT	Porphyria cutanea tarda
PeCDD	Pentachlorinated dibenzo-*p*-dioxin
PeCDF	Pentachlorinated dibenzo-*p*-dioxin

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

PEPCK	Phosphoenol pyruvate carboxykinase
PFC	Plaque-forming cell
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGST	Placental glutathione-S-transferase
PGT	Placental glutathione transferase
PHA	Phytohemagglutinin
PIQ	Performance IQ
PKC	Protein kinase C
PNS	Peripheral nervous system
POMS	Profile of Mood States
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
PRR	Prevalence risk ratio
PWM	Pokeweed mitogen
RNA	Ribonucleic acid
RR	Relative risk
SAR	Structure-activity relationships
SB-IQ	Standford Binet IQ
SCL-90-R	Self-Report Symptom Checklist-90-Revised
SD	Standard deviation
SE	Standard error
SEA	Southeast Asia
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

SIR	Standard incidence ratio
SMR	Standardized mortality ratio
SRBC	Sheep erythrocytes (red blood cells)
STS	Soft tissue sarcoma
$t_{½}$	Half-time
TBB	Tetrabromobiphenyl
TBDD	Tetrabrominated dibenzo-*p*-dioxin
TBDF	Tetrabrominated dibenzo-*p*-furan
TBG	Thyroxine-binding globulin
TBP	Thyroxine-binding protein
TCAOB	Tetrachloroazoxybenzene
TCB	Tetrachlorobiphenyl
TCDD	Tetrachlorodibenzo-*p*-dioxin
TCDF	Tetrachlorodibenzofuran
TCP	Trichlorophenol
TEF	Toxic equivalency factors
TEQ	Toxic equivalents
TGF	Thyroid growth factor
TI	T helper cell independent
TNF	Tumor necrosis factor
tPA	Tissue plasminogen activator
TPA	Tetradecanoyl phorbol acetate
TSH	Thyroid-stimulating hormone
TT	Tetanus toxoid
TTR	Transthyretrin
TxB_2	Thromboxane B_2

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

UDP	Uridine diphosphate
UDPGT	UDP-glucuronosyltransferase
URO-D	Uroporphyrinogen decarboxylase
VIQ	Verbal IQ
VLDL	Very low density lipoprotein
v/v	Volume per volume
w/w	Weight by weight
WAIS	Wechsler Adult Intelligence Scale
WISC-R	Wechsler Intelligence Scale for Children, Revised

PREFACE

In April 1991, the U.S. Environmental Protection Agency (EPA) announced that it would conduct a scientific reassessment of the health risks of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and chemically similar compounds collectively known as dioxin. The EPA has undertaken this task in response to emerging scientific knowledge of the biological, human health, and environmental effects of dioxin. Significant advances have occurred in the scientific understanding of mechanisms of dioxin toxicity, of the carcinogenic and other adverse health effects of dioxin in people, of the pathways to human exposure, and of the toxic effects of dioxin to the environment.

In 1985 and 1988, the Agency prepared assessments of the human health risks from environmental exposures to dioxin. Also, in 1988, a draft exposure document was prepared that presented procedures for conducting site-specific exposure assessments to dioxin-like compounds. These assessments were reviewed by the Agency's Science Advisory Board (SAB). At the time of the 1988 assessments, there was general agreement within the scientific community that there could be a substantial improvement over the existing approach to analyzing dose response, but there was no consensus as to a more biologically defensible methodology. The Agency was asked to explore the development of such a method. The current reassessment activities are in response to this request.

The scientific reassessment of dioxin consists of five activities:

1. Update and revision of the health assessment document for dioxin.
2. Laboratory research in support of the dose-response model.
3. Development of a biologically based dose-response model for dioxin.
4. Update and revision of the dioxin exposure assessment document.
5. Research to characterize ecological risks in aquatic ecosystems.

PREFACE (continued)

The first four activities have resulted in two draft documents (the health assessment document and exposure document) for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. These companion documents, which form the basis for the Agency's reassessment of dioxin, have been used in the development of the risk characterization chapter that follows the health assessment. The process for developing these documents consisted of three phases which are outlined in later paragraphs.

The fifth activity, which is in progress at EPA's Environmental Research Laboratory in Duluth, Minnesota, involves characterizing ecological risks in aquatic ecosystems from exposure to dioxins. Research efforts are focused on the study of organisms in aquatic food webs to identify the effects of dioxin exposure that are likely to result in significant population impacts. A report titled, *Interim Report on Data and Methods for the Assessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Risks to Aquatic Organisms and Associated Wildlife* (EPA/600/R-93/055), was published in April 1993. This report will serve as a background document for assessing dioxin-related ecological risks. Ultimately, these data will support the development of aquatic life criteria which will aid in the implementation of the Clean Water Act.

The EPA had endeavored to make each phase of the current reassessment of dioxin an open and participatory effort. On November 15, 1991, and April 28, 1992, public meetings were held to inform the public of the Agency's plans and activities for the reassessment, to hear and receive public comments and reviews of the proposed plans, and to receive any current, scientifically relevant information.

In the Fall of 1992, the Agency convened two peer-review workshops to review draft documents related to EPA's scientific reassessment of the health effects of dioxin. The first workshop was held September 10 and 11, 1992, to review a draft exposure assessment titled, *Estimating Exposures to Dioxin-Like Compounds*. The second workshop was held September 22-25, 1992, to review eight chapters of a future draft *Health Assessment Document for*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds. Peer-reviewers were also asked to identify issues to be incorporated into the risk characterization, which was under development.

In the Fall of 1993, a third peer-review workshop was held on September 7 and 8, 1993, to review a draft of the revised and expanded Epidemiology and Human Data Chapter, which also would be part of the future health assessment document. The revised chapter provided an evaluation of the scientific quality and strength of the epidemiology data in the evaluation of toxic health effects, both cancer and noncancer, from exposure to dioxin, with an emphasis on the specific congener, 2,3,7,8-TCDD.

As mentioned previously, completion of the health assessment and exposure documents involves three phases: Phase 1 involved drafting state-of-the-science chapters and a dose-response model for the health assessment document, expanding the exposure document to address dioxin related compounds, and conducting peer review workshops by panels of experts. This phase has been completed.

Phase 2, preparation of the risk characterization, began during the September 1992 workshops with discussions by the peer-review panels and formulation of points to be carried forward into the risk characterization. Following the September 1993 workshop, this work was completed and was incorporated as Chapter 9 of the draft health assessment document. This phase has been completed.

Phase 3 is currently underway. It includes making External Review Drafts of both the health assessment document and the exposure document available for public review and comment.

Following the public comment period, the Agency's Science Advisory Board (SAB) will review the draft documents in public session. Assuming that public and SAB comments are positive, the draft documents will be revised, and final documents will be issued.

PREFACE (continued)

The *Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds* has been prepared under the direction of the Office of Health and Environmental Assessment, Office of Research and Development, which is responsible for the report's scientific accuracy and conclusions. A comprehensive search of the scientific literature for this document varies somewhat by chapter but is, in general, complete through January 1994.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

This draft Health Assessment Document was prepared under the leadership and direction of the Office of Health and Environmental Assessment (OHEA) within EPA's Office of Research and Development (ORD). The overall coordination and leadership of the activities associated with EPA's reassessment of dioxin, which includes the development of this draft document, is Dr. William H. Farland, Director of OHEA.

Authors and chapter managers for the Health Assessment Document are listed below. Early drafts of some chapters were prepared by Syracuse Research Corporation under EPA Contract No. 68-CO-0043. Other chapters were authored totally or in part by scientists within EPA and other agencies within the federal government. The ORD chapter managers were responsible for providing oversight, review, and technical editing of successive drafts, and incorporating comments from reviewers to develop a comprehensive and consistent document. In some cases, the chapter managers also authored sections or parts of the chapter.

AUTHORS AND CHAPTER MANAGERS

Chapter	EPA Chapter Manager/Author	Outside Author
1. Disposition and Pharmacokinetics	Jerry Blancato U.S. EPA Environmental Monitoring Systems Laboratory Las Vegas, NV	James Olson Department of Pharmacology and Therapeutics State University of New York Buffalo, NY
2. Mechanism(s) of Action	William H. Farland U.S. EPA Office of Health and Environmental Assessment (OHEA) Washington, DC	James Whitlock, Jr. Department of Molecular Pharmacology Stanford University School of Medicine Stanford, CA
3. Acute, Subchronic, and Chronic Toxicity	Debdas Mukerjee Environmental Criteria and Assessment Office/OHEA Cincinnati, OH	Ulf G. Ahlborg Karolinska Institute Stockholm, SWEDEN

AUTHORS, CONTRIBUTORS, AND REVIEWERS (continued)

Chapter	EPA Chapter Manager/Author	Outside Author
4. Immunotoxicity	Ralph Smialowicz U.S. EPA Health Effects Research Laboratory Research Triangle Park, NC Gary R. Burleson* U.S. EPA Health Effects Research Laboratory Research Triangle Park, NC	Nancy Kerkvliet Agricultural Chemistry Oregon State University Corvallis, OR
5. Developmental and Reproductive Toxicity	Gary Kimmel U.S. EPA Human Health Assessment Group OHEA Washington, DC	Richard Peterson School of Pharmacy University of Wisconsin Madison, WI
6. Carcinogenicity of TCDD in Animals	Charalingayya B. Hiremath U.S. EPA Human Health Assessment Group OHEA Washington, DC	George Lucier National Institute of Environmental Health Sciences Research Triangle Park, NC
7. Epidemiology/Human Data Part A. Cancer Effects Part B. Effects Other Than Cancer	David L. Bayliss U.S. EPA Human Health Assessment Group OHEA Washington, DC	Charles Poole* Epidemiology Research Institute Cambridge, MA Marie Haring-Sweeney National Institute for Occupational Safety and Health Cincinnati, OH

AUTHORS, CONTRIBUTORS, AND REVIEWERS (continued)

Chapter	EPA Chapter Manager/Author	Outside Author
8. Dose-Response Modeling	Steven P. Bayard U.S. EPA Human Health Assessment Group OHEA Washington, DC	**Dioxin Dose-Response Modeling Workgroup** Michael Gallo (Co-chair), Keith Cooper, Panos Georgopolous, and Lynne McGrath UMDNJ-Robert Wood Johnson Medical School Environmental and Occupational Health Sciences Institute (EOHSI) Piscataway, NJ George Lucier (Co-chair) and Christopher Portier National Institute of Environmental Health Sciences Research Triangle Park, NC Melvin Andersen and Michael DeVito U.S. EPA Health Effects Research Laboratory Research Triangle Park, NC Steven Bayard and Paul White U.S. EPA Office of Health and Environmental Assessment Washington, DC Lorrene Kedderis University of North Carolina Chapel Hill, NC Jeremy Mills Chemical Industry Institute of Toxicology Research Triangle Park, NC Ellen Silbergeld University of Maryland Baltimore, MD
9. Risk Characterization of 2,3,7,8-TCDD and Related Compounds	William H. Farland (See Chapter 2)	

*Involved with an early draft, but no longer working on the reassessment project.

AUTHORS, CONTRIBUTORS, AND REVIEWERS (continued)

CONTRIBUTORS

Linda Birnbaum	Director, Environmental Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC
Marilyn Fingerhut	Chief, Industry-wide Studies Branch, National Institute for Occupational Safety and Health, Cincinnati, OH
Dorothy Patton	Executive Director, Risk Assessment Forum, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC
Peter W. Preuss	Director, Office of Science, Planning, and Regulatory Evaluation, U.S. Environmental Protection Agency, Washington, DC
Dwain Winters	Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC

REVIEWERS

Early drafts of Chapters 1 through 8 of this health assessment were reviewed by a panel of experts at a peer-review workshop held September 22-25, 1992. Members of the Peer Review Panel for this workshop were as follows:

Edward Bresnick	Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH
M. Judith Charles	Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC
Michael Denison	Department of Biochemistry, Michigan State University, East Lansing, MI
Phillip Enterline	Emeritus Professor of Biostatistics, University of Pittsburgh, Pittsburgh, PA

AUTHORS, CONTRIBUTORS, AND REVIEWERS (continued)

Mark Feeley	Toxicity Evaluation Division, Bureau of Chemical Safety, Health, and Welfare, Ottawa, Ontario, Canada
Thomas A. Gasiewicz	Department of Biophysics, University of Rochester, Rochester, NY
James Gillette	Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD
Claude Hughes	Duke University Medical Center, Durham, NC
Curtis D. Klaassen	Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, Kansas City, KS
Daniel Krewski	Biostatistics and Computer Applications, Environmental Health Centre, Ottawa, Ontario, Canada
Suresh Moolgavkar	Professor of Epidemiology and Biostatistics, The Fred Hutchinson Cancer Research Center, Seattle, WA
Jay Silkworth	Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY
Thomas Webster	Center for the Biology of Natural Systems, Queens College, City University of New York, Flushing, NY

On September 7 and 8, 1993, a peer-review workshop was held to review a greatly revised and expanded draft Chapter 7 (Epidemiology/ Human Data). Members of the Peer Review Panel for this workshop are as follows:

John Andrews	Associate Administrator for Science, Agency for Toxic Substances and Disease Registry, Atlanta, GA
Germaine Buck	Clinical Assistant Professor, Department of Social and Preventive Medicine, State University of New York, Buffalo, NY

AUTHORS, CONTRIBUTORS, AND REVIEWERS (continued)

Harvey Checkoway	Professor, Department of Environmental Health, University of Washington, Seattle, WA
Phillip Enterline	Emeritus Professor of Biostatistics, University of Pittsburgh, Pittsburgh, PA
M. Gerald Ott	Director of Epidemiology, BASF Corporation, Parsippany, NJ
Allan H. Smith	Professor of Epidemiology, University of California, Berkeley, CA
Anne Sweeney	Assistant Professor of Epidemiology, School of Public Health, University of Texas, Houston, TX
Karen Webb	Medical Director, HealthLine Corporation Health, St. Louis, MO

In addition, during the development of this draft Health Assessment Document, selected sections, chapters, or volumes were peer reviewed by scientists and experts within EPA and other federal agencies, as well as by experts in academia and the private sector.

A draft of Chapter 9, the risk characterization, was reviewed by an interagency workgroup comprising scientists from the following agencies of the federal government:

Department of Agriculture

Department of Defense

Department of Health and Human Services*

Department of Labor (Occupational Safety and Health Administration)

Department of Veterans Affairs

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*Drafts of Chapters 7 and 9 have been reviewed by the Subcommittee on Risk Assessment of the Committee to Coordinate Health and Environmental Related Programs (CCEHRP) under the direction of Bryan D. Hardin of the National Institute for Occupational Safety and Health, Centers for Disease Control, Department of Health and Human Services, and Ron Coene, Executive Secretary of CCEHRP.

AUTHORS, CONTRIBUTORS, AND REVIEWERS (continued)

Executive Office of the President

- Office of Science and Technology Policy
- Council of Economic Advisors
- Domestic Policy Council

CHAPTER 7. EPIDEMIOLOGY/HUMAN DATA
PART A: CANCER EFFECTS

7.1. INTRODUCTION

Animal bioassay data provide substantial presumptive evidence of the human carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (see Chapter 6), but confirmation must come from well-designed human studies. TCDD is a multiorgan carcinogen in animals. Target organs include the liver, thyroid, lung, skin, and soft tissues. There is no assumption of target tissue concordance from animals to humans, although site concordance would add support to a causal interpretation. This chapter reports on the cancer epidemiology evidence of TCDD and its congeners.

This review and analysis of the epidemiologic literature on dioxins and cancer begins by defining the scope of chemical exposures, cancers, and research reports to be considered. Then, following a brief summary of previous EPA assessments of epidemiologic literature, a description is given of the methods used in the present review. The original research reports are then discussed in four groups: 1) follow-up studies of chemical manufacturing and processing workers, 2) case-control studies in general populations, 3) studies of pulp and paper mill workers, and 4) other studies (including studies of pesticide applicators, Vietnam veterans with potential exposure to Agent Orange, residents of Seveso, Italy, exposed to TCDD during an accidental explosion, and victims of contaminated rice oil poisonings). Because the discussions of the first two groups of studies are relatively lengthy, brief summaries are given at the end of each of those sections. Conclusions are drawn following an overall discussion of all the studies.

7.2. SCOPE

Epidemiologic studies of cancer among persons exposed to TCDD and other polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are included in this review. Primary emphasis is placed on studies with exposures to TCDD itself, occurring primarily in the manufacture and use of 2,4,5-trichlorophenol, hexachlorophene, and the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Because exposures to 2,4,5-T and

2,4-dichloroacetic acid (2,4-D) often occur among the same groups who manufacture and use these herbicides, some studies of groups exposed only to 2,4-D are also included. Exposure to lower-chlorinated PCDDs (dichlorinated and trichlorinated isomers) may occur in the manufacture and use of 2,4-D. Also included are studies of groups exposed to higher-chlorinated PCDDs (i.e., the hexachlorinated, heptachlorinated, and octachlorinated isomers), occurring primarily in the manufacture and use of pentachlorophenol (PCP) and in the paper and pulp industries.

A major weakness in nearly all of these studies is the lack of good exposure information. Most studies rely solely on interviews and questionnaires of work history to ascertain exposure surrogates. There is little, if any, verification of actual internal dose to these compounds. Some studies use chloracne as a surrogate for exposure to TCDD. Three of the recent cohort studies of production workers (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990) do provide estimates of TCDD exposure in cohort samples via serum blood levels taken decades after cessation of exposure. These can be used to determine possible dose-response trends and estimate the risk of cancer to populations with low-level exposure to TCDD (see Chapter 8). Measures of exposure by individual study will be discussed.

At the time of EPA's last review in 1988, evidence of human carcinogenicity of TCDD and the phenoxy herbicides focused on soft tissue sarcomas (STSs) and malignant lymphomas. Consequently, this report will update and analyze the evidence pertaining to these cancers. But also included are evaluations of the evidence of cancer at other sites. The case-control studies reviewed for EPA's last analysis generally considered herbicide applicators with potential exposures to both 2,4-D and 2,4,5-T. Recent case-control studies of U.S. farmer groups in which exposure to 2,4-D and 2,4,5-T can be separated (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) provide a validation mechanism to separate potential effects of these herbicides and, possibly, their different PCDD contaminants. Thus, these and other recent studies (Hardell and Eriksson, 1988; Eriksson et al., 1990; Woods et al., 1987) will be reviewed and compared with those discussed in EPA's earlier reports.

Four recent cohort mortality studies (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990; Saracci et al., 1991) totaling over 23,000 workers potentially exposed to TCDD

and/or phenoxy herbicides/chlorophenols provide a new important database for analyzing cancer effects, and these will be reviewed first. The first three of these, and especially the large U.S. study of Fingerhut et al. (1991), are considered to be the most important new studies in the field of TCDD cancer epidemiology because of their attention to cohort selection and to TCDD exposures or exposure surrogates (chloracne). The fourth study (Saracci et al., 1991), while having the largest cohort, has less information on the TCDD-exposed subcohort and no information that would allow a quantitative estimate of exposure. Two other studies of phenoxy acid manufacturers are included (Lynge, 1985, 1993; Coggon et al., 1986), but their usefulness is limited due to the low unsubstantiated exposure to TCDD. Also included is a study of occupationally exposed women (Kogevinas et al., 1993) who had probable exposure to TCDD.

Three recent cohort studies of workers in the pulp and paper mill industry are included (Robinson et al., 1986; Jäppinen et al., 1987; Henneberger et al., 1989) because of potential for worker exposure to higher-chlorinated PCDDs, but not hexa-, hepta-, or octaphenoxy herbicides. However, none of these studies provide any additional information about which PCDD exposures were likely, and these studies are not given much weight.

Studies of Vietnam veterans potentially exposed to TCDD in Agent Orange are reviewed briefly, with only one (Michalek et al., 1990) judged to have sufficient information on potential TCDD exposure to be useful for analysis. Also, the recent studies of the Seveso, Italy, residents are discussed (Bertazzi et al., 1992, 1993, 1989a, b; Mocarelli et al., 1991; Pesatori et al., 1992); these studies provide some exposure data, but the cancer response analysis is limited due to inadequate follow-up time.

Finally, the studies of the rice oil poisonings of residents in Taiwan and Japan with polychlorinated biphenyl (PCB) and PCDF contaminants are reviewed (Kuratsune et al., 1988, 1975; Chen et al., 1980; Koda and Masuda, 1975; Rogan et al., 1988). Even though these poisoned oils did not contain TCDD, they did contain many TCDD-like congeners currently considered by EPA to have carcinogenic potential, which can be compared to TCDD. Also, certain dioxin-like PCBs are suspected carcinogens based on their receptor-binding characteristics and on animal studies.

Only follow-up and case-control studies are considered in this review. Case reports, other clinical observations, and prevalence surveys are excluded. The review is restricted to studies that have been published in full and that are available in the open scientific literature. Prepublication reports and studies published only in abbreviated form (as well as abstracts or letters to editors) are included only where they supplement the published articles. These restrictions limit the review to studies that have received at least a minimum of peer review and that have been described fully enough to permit a thorough assessment of materials, methods, and results.

7.3. PREVIOUS EPA REVIEWS

In the Health Assessment Document for Polychlorinated Dibenzo-*p*-Dioxins, dated September 1985 (U.S. EPA, 1985), the majority of the epidemiology studies pertained to groups of herbicide applicators with potential exposure to phenoxy acids and/or chlorophenols. In that report, the analysis emphasized case-control studies of soft tissue sarcomas and non-Hodgkin's lymphoma (NHL). That report concluded that the epidemiologic research available at that time provided "limited evidence for the carcinogenicity of phenoxy acids and/or chlorophenols in humans. However, with respect to the dioxin impurities contained therein, the evidence for the human carcinogenicity for 2,3,7,8-TCDD based on the epidemiologic studies is only suggestive because of the difficulty of evaluating the risk of 2,3,7,8-TCDD exposure in the presence of the confounding effects of phenoxy acids and/or chlorophenols." In its next report, the review draft dated June 1988 of A Cancer Risk-Specific Dose Estimate for 2,3,7,8-TCDD (U.S. EPA, 1988), the focus was essentially the same, and EPA concluded that "the human evidence supporting an association between exposure to 2,3,7,8-TCDD and cancer is considered inadequate."

7.4. REVIEW METHODS

This review will follow the spirit of the EPA Risk Assessment Guidelines of 1986 (U.S. EPA, 1987) by considering alternative explanations for results observed in epidemiologic studies. These explanations fall into the general categories of causality, chance, bias, and confounding. The basic approach is akin to a process of elimination, by

which one attempts to determine the direction and to quantify the magnitude of the influence that chance, bias, and confounding may have had on the results of each study. Wherever possible, the results of all studies will be reported in units of relative risk estimates and 95% confidence limits.

Most biases in epidemiologic studies can be placed into one of two categories: biases of classification and biases of selection. Classification biases can result from inaccurate ascertainment of exposure, disease, or confounders. Selection biases can result from nonrepresentative sampling of populations, as in the selection of controls in case-control studies, or from incomplete participation by study subjects. Any bias gains tenability as an explanation for an observed result if empirical evidence can be adduced to buttress the mere suggestion that the bias might have occurred. Only those biases considered to be potentially important will be addressed explicitly in this review.

When imprecise exposure estimates are available, such as with much of the epidemiologic data on dioxin, estimates of risk can be potentially biased toward the null. Misclassification, if random, could potentially lead to a masking of a true effect.

Classification biases are of two kinds: differential versus nondifferential. Differential misclassification will lead to either an exaggeration or an underestimation of an effect. Nondifferential misclassification occurs when the exposure or disease classification is incorrect in a portion of the subjects (cases or controls). This type of bias is generally toward the null, and the risk estimate may reflect this. This can happen when some subjects are classified as having exposure to dioxin when they really were not exposed. Similarly, some actually may have had exposure but were classified as not having had it. In studies where few or no effects were seen, researchers must seriously consider the problem of nondifferential misclassification. This can be the reason why nonpositive risk estimates or even disparate risk estimates are seen from different studies of the same effect. On the other hand, in studies with significant results, nondifferential misclassification is not likely to be a cause of a significant finding.

Recall bias may produce the opposite effect. Persons with a disease may tend to remember exposure to a substance better when it is known to them that such exposure might be associated with the disease. This could potentially lead to inflated risk estimates. In fact,

it has been suggested that such biases are present in many of the case-control studies on dioxin. The Swedish studies by Hardell and colleagues have been particularly singled out for criticism in this respect. That some recall bias may be present is confirmed in a later case-control study (Hardell and Eriksson, 1988) in which some reduction of risk estimates was produced by the use of cancer controls.

Confounding bias is a tenable explanation for an association between an exposure and a disease if the hypothetical confounder can be named and if a good case can be made that it is a cause of the disease, that it was associated with the exposure in the study population, and that it was not adequately controlled in the study design or data analysis. This review will explicitly mention only those potential confounders that meet all of these criteria.

As stressed in previous EPA reviews (1985, 1988), concomitant exposures present a special problem of potential confounding in the literature on TCDD and related chemicals. As a noteworthy example, an association between 2,4,5-T exposure and a given cancer, if causal, could be due to 2,4,5-T itself, to TCDD, or to some other contaminant. The problem multiplies when it is recognized that, historically, many phenoxy acid herbicide preparations were mixtures of 2,4,5-T and 2,4-D and that many persons who manufactured, processed, and used these preparations were exposed to other chemicals as well. Nevertheless, it may be possible by examining studies of persons exposed to different combinations of chemicals to identify "threads" of commonality and differences in the results, especially when specific cancers are considered separately.

Publication bias, sometimes considered a form of selection bias, is the tendency for the results of a study to influence a judgment as to whether or not it will be published. The direction and magnitude of publication bias is difficult, if not impossible, to quantify. It is expected to be a much greater problem in literature reviews and in studies relying on existing records than in original research in which substantial resources are devoted to collection of data of relatively high quality. The level of effort required for such studies creates a strong incentive to publish the results. There is a tendency to publish studies with positive results as opposed to studies with nonpositive findings.

Strength of association, as measured by the magnitude of the estimated relative risk, is an important feature of a study's results. The stronger the association, the stronger a bias

or confounding factor would have to be to explain it. Because questions of bias and confounding are study-specific, no defensible criteria can be set up in advance to place relative risk values into categories of strength of association.

Trends in increased risk by degree of exposure and by time since first exposure (latency) are also important. Different hypothetical causal mechanisms might predict different exposure-response and latency patterns. Hypotheses of steadily increasing effect with increasing exposure (i.e., monotonic exposure-response functions) and hypotheses of effects early in the carcinogenic process (e.g., for factors that operate at the initiation stage) predict that increases in risk will be greatest among persons with relatively high degrees of exposure and after relatively long latency periods. Hypotheses of tumor promotion and/or initiation will be discussed in the appropriate sections.

Replication of results is important in all scientific research. When several studies have shown a positive association of effect with the same exposure but were conducted under different circumstances, the possibility that an unknown confounder or chance produced the observed elevated effect is minimized. When different investigators working with different populations using different methods confirm an original finding, the results are more believable.

The statistical aggregation of results from different studies (meta-analysis) has become a popular feature of epidemiologic literature reviews. In this review, results from separate studies are aggregated only when all key methodologic features and results are reasonably similar. The method of aggregation used here is to take the ratio of the sum of the cause-specific observed deaths to the sum of the cause-specific expected deaths for the individual studies. Because investigators recognize the value of varying their methods to test methodologic hypotheses, and because results often differ appreciably, aggregation of results is not often indicated and is done here with caution.

7.5. FOLLOW-UP STUDIES OF CHEMICAL MANUFACTURING AND PROCESSING WORKERS

7.5.1. United States

Fingerhut and colleagues (1990, 1991) reported a study of 5,172 males who had worked at 12 plants in the United States in the production of chemicals contaminated with TCDD. Five thousand of the cohort members (97%) were identified in company records as having been "assigned to a production or maintenance job in a process involving TCDD contamination" (Fingerhut et al., 1991). The remaining 172 cohort members were "identified in a previously published study on the basis of exposure to TCDD" (Fingerhut et al., 1991). This cohort subsumed, and thereby supplanted, company-specific cohorts from Dow Chemical USA (Ott et al., 1987; Cook, 1981) and the Monsanto Company (Zack and Gaffey, 1983; Zack and Suskind, 1980) that had been the subject of previous reports. This study was initiated in 1978 to determine whether health effects were apparent in humans who were exposed to 2,4,5-T. In 1978, toxicological, teratogenic, and carcinogenic effects data were released that indicated a cancer effect in animals. There was a concern about the potential effects of exposure to Agent Orange on Vietnam veterans and workers who produced products that were contaminated with dioxin (Fingerhut et al., 1992). Follow-up began in 1940 or on the date of the "first systematically documented assignment to a process involving TCDD contamination" (Fingerhut et al., 1991), whichever was later, and closed at the end of 1987. Comparisons were made with the United States population.

The authors stated that approximately 13% of the cohort of 5,172 workers had records of chloracne. The presence of chloracne in a group of people is an indicator of relatively intense exposure to TCDD. It can be caused by higher-chlorinated PCDDs, PCDFs, and PCBs as well (O'Malley et al., 1990). It is a highly specific indicator of exposure because it virtually never occurs among unexposed persons. It is a nonsensitive indicator, however, because many highly exposed persons do not develop it (Manz et al., 1991; Caramaschi et al., 1981; Mocarelli et al., 1991). Exposure to other dioxin-like chemicals that can be found in the workplace may produce a form of chloracne that could be indistinguishable from that produced by dioxin (Ott et al., 1993). These chemicals can be dioxin-like in their effects and act through the aryl hydroxylase (Ah) receptor.

Although all members of the cohort had specific assignments to TCDD exposure areas in common, exposures to multiple chemicals are the rule rather than the exception in the chemical industry. At one plant, for instance, considerable overlap existed among persons involved in the production of chlorophenols, 2,4,5-T, and 2,4-D (Ott et al., 1987; Bond et al., 1988, 1989a), and who were thus exposed to TCDD and higher-chlorinated and lower-chlorinated PCDDs. Presumably, many persons throughout the cohort had contact with substantial numbers of other chemicals. Although comprehensive surveys of chemical exposures were conducted in plant-specific cohorts, the results are not available.

Special attention was paid to results for the 3,036 workers who were followed for at least 20 years after first exposure. This group was again divided into those with less than 1 year (N=1,516) and those with more than 1 year (1,520) of exposure (referred to below as the long duration/latency subcohort). One year was chosen as the criterion for duration of exposure because an analysis of 253 workers from 2 plants showed that every worker with 1 or more years of exposure had a lipid-adjusted serum TCDD level greater than the mean value (7 ppt) in a comparison group of unexposed workers (Fingerhut et al., 1990). Although the average level for all 253 workers was 233 ppt, the average increased to 418 ppt in those 119 who were exposed for 1 or more years (Fingerhut et al., 1991). The researchers described a plan to replace this duration-based exposure scale by using "a dioxin exposure matrix constructed from historic process descriptions, analytic measurements of TCDD and industrial hygiene data . . . to develop the relative ranking of workers exposed to TCDD" (Fingerhut et al., 1990).

The cohort as a whole experienced an estimated 15% (95% CI=1.0-1.3) elevation of mortality from all cancers combined, with a 46% elevation (95% CI=1.2-1.8) among those in the long duration/latency subcohort (Table 7-1). An excess of deaths from cancers of connective and soft tissues (STSs) was apparent in the total cohort (RR=3.4, CI=0.9-8.6) and in the long duration/latency subcohort (RR=9.2, CI=1.9-27.0), but these results were based on only four deaths and three deaths, respectively, from two different plants. A 40% overall elevation in deaths (CI=0.7-2.5) from non-Hodgkin's lymphoma was confined to workers in the total cohort and was not seen in the long duration/latency subcohort. Results for Hodgkin's disease were highly imprecise, based on only three deaths (vs. 2.5 expected)

Table 7-1. Relative Risks of Selected Cancers in Study of Chemical Manufacturing Workers Exposed to TCDD in United States, by Exposure Duration and Latency

Cancer	Measure[a]	Latency <20 years	Latency ≥20 years		Total cohort
			Exposure <1 year	Exposure ≥1 year	
Connective and soft tissues	Observed deaths	1	0	3	4
	Relative risk	1.4	0.02	9.22	3.4
	95% confidence interval	0.1 - 7.0	0.0 - 15.0	1.90 - 27.0	0.9 - 8.6
Hodgkin's disease	Observed deaths	2	0	1	3
	Relative risk	1.1	0.0	2.8	1.2
	95% confidence interval	0.2 - 3.5	0.0 - 15.0	0.1 - 15.3	0.3 - 3.3
Non-Hodgkin's lymphomas	Observed deaths	6	2	2	10
	Relative risk	1.6	1.5	0.9	1.4
	95% confidence interval	0.7 - 3.4	0.2 - 4.9	0.1 - 3.30	0.7 - 2.5
Lung cancer	Observed deaths	32	17	40	89
	Relative risk	0.9	1.0	1.4	1.1
	95% confidence interval	0.7 - 1.3	0.6 - 1.5	1.0 - 1.9	0.9 - 1.4
Stomach cancer	Observed deaths	3	3	4	10
	Relative risk	0.6	1.8	1.4	1.0
	95% confidence interval	1.5 - 1.6	0.4 - 5.2	0.4 - 3.5	0.5 - 1.9
All combined	Observed deaths	103	48	114	265
	Relative risk	1.05	1.02	1.46	1.15
	95% confidence interval	0.8 - 1.2	0.76 - 1.4	1.2 - 1.8	1.0 - 1.3

[a]Relative risks and confidence intervals are based on rounded values and may differ slightly from those in the original reports (Fingerhut et al., 1990, 1991).

Source: Fingerhut et al., 1991.

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in the total cohort. Lung cancer was elevated by 10% overall but by 40% (CI=1.0-1.9) in the long duration/latency subcohort. A similar 40% excess of stomach cancer (CI=0.4-3.5) in this subcohort was based on only four deaths; no excess was seen in the total cohort.

The investigators conducted a special study of connective and soft tissue cancers. A review of all available hospital records and tissue specimens failed to confirm the indications of soft tissue sarcomas on two of the four death certificates that had been assigned to this cause-of-death category (Fingerhut et al., 1990). The review also provided evidence that two persons in plant 8 whose deaths had been assigned to other causes of death had actually had soft tissue sarcomas. Because only the exposed cohort's death certificates were subjected to detailed review, the analytic comparisons with the United States population were required to be based strictly on death certificate information. The basic and well-known rule in such situations is that, absent evidence to the contrary, erroneous information on death certificates must be considered to have been equally frequent in the two groups being compared. Suruda et al. (1993), in a study of STS diagnoses in cohorts exposed to dioxins and chlorinated naphthalenes, found that death certificates are "relatively insensitive" for detecting STS and that the power of life table analysis to detect excess risks of STS may be reduced compared with its utility in correctly estimating the risk of other cancers, such as colon cancer or rectal cancer. However, the correct identification of STS as the underlying cause of death on death certificates appears to be much better (82%), based on medical records, than first thought according to the results of this study. Medical records on the remaining 18% could not be found. If a death certificate gives as an underlying cause of death a soft tissue sarcoma and it is coded as 171x, it is very likely correctly coded according to the authors. In an earlier study, this figure was estimated by Percy et al. (1981) to be 55%.

Four of the soft tissue sarcomas that are discussed in Fingerhut's study (two are included in Fingerhut's life table analysis while two others are discussed but did not qualify for inclusion in the life table analysis) are actually from the Nitro, West Virginia, plant. The remaining two cases that were included in Fingerhut's life table analysis are from a different plant in the study. One of these cases also suffered chloracne. The four cases from the Nitro, West Virginia, plant are also the subject of a later study by Collins et al. (1993). The two cases included in Fingerhut's life table analysis had previously suffered chloracne from

the 1949 accident (Zack and Gaffey, 1983) in which 121 workers developed chloracne as a result of a trichlorophenol process accident on March 8, 1949. Collins included one more STS not previously discussed by Fingerhut from the same plant. The Collins study is reviewed later.

If the properly diagnosed STSs were correctly assigned to their appropriate plants and the two incorrectly diagnosed STSs were removed, there would be one in plant 9 and three in plant 8. No STSs were found at the other plants. This unusually skewed distribution might possibly be related to the accident that happened in plant 8 some 44 years earlier. Or perhaps physicians in Nitro, West Virginia, are more likely to diagnose STS than are physicians in other areas of the country. Because of the rarity of STS, there was inadequate statistical power to expect to see STS in the other individual plants. If this cancer has a long latent period and an excess risk is associated with exposure to 2,3,7,8-TCDD, then it might be several more years before this cohort will produce STSs at the other facilities that are part of the study.

In any case, this nondifferential misdiagnosis can potentially bias risk estimates downward.

Cases of soft tissue sarcoma include a diverse group of histological entities. All soft tissue sarcomas arise only from mesenchymal tissue and all share common features that make them alike in their basic intercellular and intracellular composition rather than different in their morphology and location. Characterizing them as fundamentally different because they are found in different sites of the body is perhaps inadequate for the determination of the risk of cancer (Enzinger and Weiss, 1988).

The histological classification of STS is centered on a dozen distinctly different classes of mesenchymal cells that form six relatively well-defined but widely distributed organ systems. By considering the growth pattern and cell morphology with an evaluation of intracellular and extracellular products of the tumor cells, fairly precise histogenetic classification of soft tissue sarcomas is possible (Hajdu, 1981). For human cancer risk assessment, all connective tissues developing from the same mesodermal tissue, expressing the same set of "proto-oncogenes" and surrounded by the same chemical milieu of the extracellular matrix, are expected to develop cancer following exposure to certain

carcinogens. The grouping of these end-target organs together is therefore a necessary measure to evaluate 2,3,7,8-TCDD human carcinogenicity.

Confounding by cigarette smoking must be considered in interpreting the approximate 40% excess of lung cancer deaths in the long duration/latency subcohort (Table 7-2). For the United States as a whole, the authors (Fingerhut et al., 1990) computed age-adjusted proportions of 24% never smokers, 19% former smokers, and 57% current smokers in 1965 (roughly midway through the follow-up period). The corresponding proportions were 28% never smokers, 14% former smokers, and 59% current smokers among the 87 workers from the study of serum TCDD levels who were members of the long duration/latency subcohort as well. Assuming relative risks of lung cancer of 4.7 for former smokers and 10.9 for current smokers, the authors used a standard technique (Axelson and Steenland, 1988) to adjust the number of expected lung cancer deaths and found essentially no change in the results. It should be kept in mind that the sample of smoking histories was taken from only 2 plants, numbers 1 and 2, but the excess in lung cancer risk was chiefly in plants 8 and 10. The generalization of smoking habits of employees in 2 of the 12 participating plants to that of the entire cohort may not be representative of the true smoking impact of the risk of lung cancer to the entire cohort. Most of the lung cancers came from 3 facilities (56 of 89 observed lung cancer deaths), plant numbers 8, 9, and 10. The remaining 7 plants contributed the remaining 33 lung cancers to the total due to the small sizes of the respective subcohorts and insufficient latency. It then would be possible to evaluate the effect of smoking on the risk of lung cancer at each individual plant. It should be remembered that national U.S. rates were used to derive expected deaths in each of the 12 plants. It is possible that local or regional rates at the locality of each of the plants may be a more appropriate comparison population for the cancer sites examined by the authors, although local rates may be unstable. In addition, if biomonitoring could be extended to the remaining 10 plants, a better idea could be derived concerning the dose levels that are associated with those plants experiencing higher lung cancer rates. The authors point out that deaths from other diseases associated with smoking such as diseases of the heart and circulatory system were either not increased or significantly decreased in this cohort (Fingerhut et al., 1990). This led the authors to conclude that "cancers of the respiratory tract . . . may result from

Table 7-2. Relative Risks of Lung Cancer in Subcohort of Chemical Manufacturing Workers Exposed to TCDD in United States for at Least 1 Year and With at Least 20 Years Latency, Adjusted for Alternative Hypotheses About Its Smoking Distribution

Proportion of subcohort (percent)[a]			Expected lung cancer deaths (40 observed)	Relative risk	95% confidence interval
Never smokers	Former smokers	Current smokers			
24	19	57	28.8	1.4	1.0 - 1.9
28	14	59	29.2	1.4	1.0 - 1.8
25	10	65	30.5	1.3	0.9 - 1.8
20	15	65	31.2	1.3	0.9 - 1.7
15	15	70	33.2	1.2	0.9 - 1.6
10	20	70	33.9	1.2	0.9 - 1.6
15	10	75	34.4	1.2	0.8 - 1.6
10	15	75	35.1	1.1	0.8 - 1.5

[a]The first set of proportions assumes no difference between the subcohort and the United States population. The second set is based on 87 surviving members of the subcohort (Fingerhut et al., 1990). The remaining sets are hypothetical values used to test the sensitivity of the results. Relative risks of lung cancer are assumed to be 4.7 for former smokers and 10.9 for current smokers (Fingerhut et al., 1990).

Source: Fingerhut et al., 1991.

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exposure to TCDD, although we cannot exclude the possible contribution of factors such as smoking and occupational exposure to other chemicals" (Fingerhut et al., 1991).

One possible explanation for the increase follows from the fact that the comparison of smoking habits between the United States population and the 87 surviving members of the long duration/latency subcohort did not show the well-known tendency for smoking to be more common among blue-collar workers than in the general population. A possible explanation for this result is that because smoking appreciably elevates the overall death rate, fewer and fewer smokers will remain in a fixed group of persons as time goes by. Thus, the use of the 87 *surviving* members of the long duration/latency subcohort may have underestimated the proportions of former and current smokers in the subcohort as a whole over the course of mortality follow-up. However, this same phenomenon is also present in the population from which expected deaths were generated, so the effect is probably nullified. As shown in Table 7-2, the actual proportions would not have to be inordinately high for smoking to have exerted appreciable confounding on the estimated relative risks of lung cancer. The lack of increased mortality from cardiovascular diseases as well as cancer of the buccal cavity and pharynx in this cohort, however, makes this explanation less likely. Furthermore, as the authors point out, mortality from nonmalignant respiratory disease (standard mortality ratio [SMR] = 96), which is often associated with smoking, was less than expected.

On the other hand, the authors report a correlation coefficient of 0.72 between length of exposure and serum TCDD tissue level. This negative bias is potentially much greater than the positive bias that could possibly be produced by smoking. And because nondifferential bias is the only type of bias that could occur in the Fingerhut study, risk estimates are more than likely to be lower than the true risk. The only way that dioxin exposure could have a positive bias is if it prevents cancer and the exposure classification is 100% wrong.

The authors of this study also report that two of these cancer deaths were of mesothelioma, a finding that indicates more than likely exposure to asbestos. Although these two mesotheliomas occurred at plants 9 and 12, it is not known whether these persons were exposed in the current job or perhaps in some previous job. If exposure occurred throughout

this cohort, it is possible that asbestos also could be a confounding factor for lung cancer. The combination of smoking and exposure to asbestos fiber might have a synergistic confounding effect on the risk of lung cancer.

The positive association of lung cancer in male workers observed in this study is also consistent with an excess of pulmonary tumors found in male mice and rats exposed to TCDD (see Chapter 6). These same animal data suggest the possibility of a protective hormonal effect from TCDD and the risk of pulmonary cancer in female rats. Because this study dealt with only male workers, this hypothesis could not be verified in female workers. On the other hand, no elevated risk of liver cancer is evident in male workers even in the long duration/latency subcohort. This is also consistent with rat data where the tumors were only observed in female rats. If there is a promoting effect on liver cancer in females due to hormonal effects as suggested by the rat studies, it could not be verified.

In a recently published paper (Collins et al., 1993), it is suggested that there is an association of STS with exposure to 4-aminobiphenyl. The authors reported that workers who developed chloracne from an accident in which a chemical mix containing TCDD was scattered throughout a 2,4,5-trichlorophenol plant had increased mortality from soft tissue sarcoma, bladder cancer, and respiratory cancer. All individuals who were identified in this study of 754 chemical employees as having STSs or lung cancers, and who were employed at the time of the 1949 accident, were potentially exposed to 4-aminobiphenyl as well as to 2,3,7,8-TCDD. However, it is not known how many were actually working inside the plant when the accident occurred. 4-Aminobiphenyl is thought to be a bladder carcinogen from previous studies. However, this chemical has not been shown to be associated with STS or lung cancer in humans. Unfortunately, no tissue measurements are available to substantiate exposure to 2,3,7,8-TCDD or exposure to 4-aminobiphenyl. It is also of interest that no significant increase in STSs was noted in the "chloracne-free" subgroup exposed to 4-aminobiphenyl. However, the authors report that an additional 106 persons also had indications of chloracne-type conditions noted in their medical files, presumably as a result of exposure to 2,3,7,8-TCDD and not as a result of the accident. Although these individuals probably were heavily exposed to dioxin as well, they were included in the "no chloracne" subgroup for the purpose of analysis. It would be of some interest to see how much of an

effect would occur to the risk estimates if they were included in the "chloracne" subgroup. Furthermore, plant employees who left work before March 8, 1949, or began work after November 22, 1949, were not included even if they had received exposure to dioxin or even developed chloracne as a result of exposure. The major interest according to the authors was in the 122 workers who developed chloracne from the 1949 accident. The authors point out that the numbers are small and that confounding factors, such as misclassification of exposure, cannot be ruled out. This study presents an interesting theory that needs to be investigated further. However, at this time, it has not been substantiated anywhere.

It was also noted by Collins that toxicological evidence is available that supports the idea that STSs (i.e., angiosarcomas) have resulted from exposure to 4-aminobiphenyl (Schieferstein et al., 1985). Angiosarcomas and bladder cancer in females were found to be dose-related with oral consumption of 4-aminobiphenyl in drinking water. However, angiosarcomas of the liver arise mainly from the endothelial lining of blood vessels. This type of tissue is more likely to be susceptible to a hydrophilic carcinogen such as 4-aminobiphenyl during its passage through the blood vessel. Hydrophobic carcinogens such as 2,3,7,8-TCDD might be expected to exert an influence on mesenchymal tissue from which most STSs arise, i.e., fibrosarcoma, histiocytoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, synovial sarcoma, schwannoma, myxoid neurogenic sarcoma, and others. Therefore, the author's assumption that 4-aminobiphenyl can cause other types of STSs remains unproven and highly unlikely.

7.5.2. Germany

Manz and colleagues (1991) reported a study of 1,583 persons (1,184 men and 399 women) employed at a German chemical manufacturing facility that produced 2,4,5-T and its precursor, 2,4,5-trichlorophenol. In 1954, a chloracne outbreak had occurred in the working population of the plant, and after that, production of the TCDD contaminant was reduced. Cohort members worked at least 3 months from 1952 through 1984. The start of follow-up was not stated in the report, but presumably began on the date of accumulation of 3 months of employment. The follow-up period closed at the end of 1989. The cohort's mortality experience was compared with that of the West German population and with that of a cohort

of workers at a gas supply company. Because the results did not differ materially between the analyses, and because limited data on the gas workers forced that comparison to be based on a subset of the TCDD-exposed cohort, only the results of the comparisons with West Germany are reported here.

The cohort was postdivided by duration of employment and by a three-category exposure scale based on TCDD measurements "in nonsystematic samples of precursor materials, products, waste, and soil from the grounds of the plant, mainly after the plant had closed in 1984" (Manz et al., 1991). This scale was validated to some extent by adipose tissue TCDD levels in 48 volunteers (mean = 296 ng/kg in 37 persons from the highest group, 83 ng/kg in 11 persons from the other two groups). Based on these results, the low and intermediate groups are combined for the present analysis.

For the males, this study, with 75 total cancer deaths expected and 24 expected in the high-exposure subcohort, was considerably smaller than the study by Fingerhut et al. (1991), which had 230 total cancer deaths expected and 78 in its long duration/latency subcohort (Table 7-3). Manz et al. presented detailed analyses only for all cancers combined. The high-exposure subcohort, and especially those with longer employment duration, experienced an excess of total cancer deaths (RR=1.4, CI=1.0-2.0 for the high-exposure group and RR=2.6, CI=1.2-4.9 for the high exposed/long duration subcohort) (Table 7-3). The authors concluded that "the increase in (total) cancer risk of 1.24-1.39 . . . cannot be explained completely by confounding factors, and . . . is associated with exposure to TCDD" (Manz et al., 1991). In a later abstract of an update of this same paper, Dwyer (1992) reported that using a Cox regression analysis of nine major areas of employment within the plant, the area of work with the strongest relative risk for cancer mortality was found to be in 2,4,5-T production (RR=2.7, CI=1.7-4.2). These findings are similar to those of Fingerhut et al. (1991).

For the cohort as a whole, the estimated relative risk of lung cancer was 1.4 (CI=1.0-2.0, 30 observed deaths). Smoking as an explanation for the observed increase in lung cancer mortality is less likely because a comparison using the gas worker reference actually leads to an increased RR of 1.7 (CI=1.1-2.4). Although smoking histories were not available for the entire Boehringer cohort, of the 361 men, 73% reported that they smoked.

Table 7-3. Relative Risks of All Cancers Combined in Study of Chemical Manufacturing Workers Exposed to TCDD in Germany, by Duration and Category of Exposure

		Exposure category (median adipose TCDD level)		
Exposure duration	Measure	Low and medium (60 ng/kg)	High (137 ng/kg)	Total
<20 years	Observed deaths	49	26	75
	Relative risk	1.1	1.2	1.1
	95% confidence interval	0.8 - 1.4	0.8 - 1.8	0.9 - 1.4
≥20 years	Observed deaths	10	8	18
	Relative risk	1.5	2.6	1.9
	95% confidence interval	0.8 - 2.7	1.2 - 4.9	1.1 - 2.9
Total	Observed deaths	59	34	93
	Relative risk	1.2	1.4	1.2
	95% confidence interval	0.9 - 1.5	1.0 - 2.0	1.0 - 1.5

Source: Manz et al., 1991.

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Similarly, 76% of 2,860 gas workers smoked. Substantial confounding based on smoking does not appear to be present because smoking seems to be similar in both plants. The estimate for stomach cancer was 1.2 (CI=0.7-2.1, 12 observed deaths). Three deaths from non-Hodgkin's lymphomas and no connective and soft tissue cancer deaths were observed. (The authors described an additional three deaths from chronic lymphocytic leukemia as non-Hodgkin's lymphoma deaths, but these deaths would not have been classified as non-Hodgkin's lymphomas in the other studies in this review.) Expected numbers of deaths from these cancers were not given. Based on the proportions of expected cancer deaths in the Fingerhut study, one might estimate that approximately 2.4 non-Hodgkin's lymphoma deaths and 0.4 connective and soft tissue cancer deaths would have been expected in this cohort as a whole and about 0.1 connective and soft tissue cancer deaths in the high-exposure subcohort. (The numbers of expected deaths from lung cancer, stomach cancer, or non-Hodgkin's lymphoma in the high-exposure subcohort were not estimated because information is lacking on how many of the observed deaths from these cancers were in that subcohort.)

The authors reported exposure to other industrial chemicals, such as benzene and dimethylsulphite. In addition, manual laborers were exposed to asbestos "probably" to some extent. However, the authors maintain that this exposure explains neither the increased mortality from all cancers nor the patterns of associations with TCDD exposure groups.

Other possible sources of bias include a potential lack of comparability between cause of death ascertainment based on medical records in some, perhaps many, cohort members versus use of death certificates only for cause of death certification in the derivation of German national death rates. This is somewhat alleviated by the use of gas workers as a second comparison group. In these workers, the same methods were used for medical certification, making comparison of cause of death somewhat more comparable. However, this is offset by the fact that the gas workers may have somewhat better mortality experience because they had to work a minimum of 10 years in order to obtain entrance into the gas workers cohort, whereas the dioxin cohort had to work only a minimum of 3 months. This could have introduced survivorship bias in this group and consequently lower mortality and higher risk estimates when compared with dioxin workers.

Furthermore, the lack of an analysis of the mortality data by time since first exposure for individual causes such as lung cancer makes it impossible to assess latent effects.

Of the 399 female cohort members, only 7% worked in high-exposure departments. In total, there were 54 deaths and an overall RR=0.8 (CI=0.6-1.0). The RR for all cancers was 0.9 (CI=0.6-1.4), but the RR=2.2 (CI=1.0-4.1) for breast cancer was significantly increased based on 9 deaths. This is an interesting result in view of a suggestion of reduced mammary cancer based on mechanistic studies and animal bioassays. However, at this point the data do not provide a sufficient basis for any conclusions. Of all the worker follow-up studies with TCDD, this is the only one that reports on a cohort of females.

In another investigation in Germany, Zober and colleagues (1990) studied persons employed at a German chemical manufacturing facility where 2,4,5-trichlorophenol was produced. An uncontrolled decomposition reaction in 1953 and subsequent cleanup activities resulted in substantial TCDD exposures. The cohort contained 247 persons who had worked at the plant from 1953 through 1987, 51% of whom had developed chloracne or erythema (a skin condition suggestive of chloracne), with mortality follow-up covering the same calendar period. Seventy-eight persons had died, RR=0.95; 23 had died of cancer, RR=1.2 (CI=0.8-1.7). Expected deaths were based on national mortality rates in the Federal Republic of Germany. When workers with chloracne were looked at separately, the risk of cancer as expressed by the SMR rose to 1.4 (CI=0.9-2.1). Again, within this highly exposed subgroup, if the analysis is restricted to only those workers who were observed 20 or more years after first employment, the SMR was significant at 2.0 (CI=1.2-3.2). For lung cancer, the SMR is of borderline significance at 2.5 (CI=1.0-5.3). The authors report that the results ". . . do not support a strong association between cancer mortality and TCDD, but they do suggest that some hazard may have been produced."

Three subcohorts were defined on the basis of procedures by which cohort members were identified as having potential for varying degrees of exposure. Subcohort C1 contained 69 persons known to be exposed to TCDD during the accident period. Cohorts C2 (84 persons) and C3 (94 persons) contained workers thought to be exposed to lesser amounts of TCDD. Recent TCDD levels in blood samples from small numbers of persons in each group suggested that exposures had been higher in C1 (median 24.5 ppt, 11 samples) than in C2

(median 9.5 ppt, 7 samples) or C3 (median 8.4 ppt, 10 samples). Thus, C2 and C3 are grouped together in this review. A separate analysis by the authors divides the group into the 127 persons with chloracne (N=114) and erythema (N=13) versus those 120 persons with neither. The average serum TCDD levels in the two subcohorts are 15 ppt and 5.8 ppt, based on 16 samples (with chloracne) and 12 samples (without chloracne), respectively. The two stratifications provide similar results. Only the first is presented here. Section 8.5 provides an additional analysis.

This study, with only 20 expected cancer deaths in the total cohort and 4 expected cancer deaths in the members of subcohort C1 with 20 or more years of latency, is much smaller than the studies by Fingerhut et al. (1991) and Manz et al. (1991). The authors, however, did provide detailed analyses of data on specific cancers (Table 7-4). Elevated mortality rates from lung cancer, stomach cancer, and all cancers combined were confined largely to the members of subcohort C1 with long latency. The confidence intervals for the relative risk estimates are extremely wide, however. No deaths from cancers of connective and soft tissues or from non-Hodgkin's lymphomas were observed, and expected numbers of deaths from these cancers were not reported. However, one mesothelioma was reported in a plant supervisor with known asbestos exposure. Based on the proportions of all expected cancer deaths due to these cancers in the study by Fingerhut et al. (1991), one might estimate that approximately 0.6 non-Hodgkin's lymphoma deaths and 0.1 connective and soft tissue cancer deaths would have been expected in this cohort as a whole, and about 0.2 non-Hodgkin's lymphoma deaths and less than 0.1 connective and soft tissue cancer deaths among the members of subcohort C1 with long latency. This study lacks power to detect a significant site-specific cancer risk at most sites due to its small size.

7.5.3. Ten-Country Study by International Agency for Research on Cancer

A historical cohort study of cancer mortality in 18,390 production workers or sprayers exposed to chlorophenoxy herbicides and/or chlorophenols was reported on by Saracci et al. (1991). Exposure was reconstructed through questionnaires, factory or spraying records, and job histories. Workers were classified as exposed (N=13,482), probably exposed (N=416), exposure unknown (N=541) and nonexposed (N=3,951). The

Table 7-4. Relative Risks of Selected Cancers in Study of Chemical Manufacturing Workers Exposed to TCDD in Germany, by Median Blood TCDD Level and Latency

Subcohort (median blood TCDD level)	Cancer	Measure	Time since first exposure <20 years	Time since first exposure ≥20 years	Total
C1 (24.5 ppt)	Lung	Observed deaths	1	3	4
		Relative risk	1.2	2.5	2.0
		95% confidence interval	0.1 - 6.2	0.6 - 6.9	0.6 - 4.8
	Stomach	Observed deaths	1	2	3
		Relative risk	2.0	4.0	3.0
		95% confidence interval	0.1 - 9.7	0.7 - 13.2	0.8 - 8.1
	All combined	Observed deaths	2	7	9
		Relative risk	0.7	1.7	1.3
		95% confidence interval	0.1 - 2.4	0.7 - 3.3	0.6 - 2.4
C2 (9.5 ppt) and C3 (8.4 ppt)	Lung	Observed deaths	0	2	2
		Relative risk	0.0	1.0	0.5
		95% confidence interval	0.0 - 1.8	0.2 - 3.4	0.1 - 1.8
	Stomach	Observed deaths	0	0	0
		Relative risk	0.0	0.0	0.0
		95% confidence interval	0.0 - 3.2	0.0 - 3.9	0.0 - 1.7
	All combined	Observed deaths	5	9	14
		Relative risk	0.8	1.3	1.1
		95% confidence interval	0.3 - 1.9	0.6 - 2.4	0.6 - 1.8
Total cohort	Lung	Observed deaths	1	5	6
		Relative risk	0.4	1.6	1.1
		95% confidence interval	0.0 - 2.0	0.6 - 3.5	0.4 - 2.2
	Stomach	Observed deaths	1	2	3
		Relative risk	0.7	1.6	1.1
		95% confidence interval	0.0 - 3.4	0.3 - 5.2	0.3 - 3.0
	All combined	Observed deaths	7	16	23
		Relative risk	0.8	1.5	1.2
		95% confidence interval	0.4 - 1.6	0.9 - 2.3	0.8 - 1.7

Source: Zober et al., 1990.

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exposed group contains everyone known to have sprayed chlorophenoxy herbicides and everyone who had worked in any of certain specified departments at factories producing chlorophenoxy herbicides. The criteria for duration or level of exposure required for selection was reported for only 3 of the 10 countries and only 4 of the 20 cohorts; these ranged from at least 1 month to 1 year. For all the other cohorts, the criterion for inclusion was to have ever been employed in production or spraying of these herbicides. The cohort contained 1,537 female workers, but results were not presented separately, except for female breast and genital organ cancers by phenoxy herbicide exposure. Average follow-up for the cohort was 17 years; 5% of eligible workers were lost to follow-up. Three of the cohorts comprising over 10,000 workers have also been reported in separate publications but for different follow-up periods (Lynge, 1985, 1987, 1993; Coggon et al., 1986; Kogevinas et al., 1993); these are discussed briefly in this report following this discussion.

Also included in the analysis was a division of the cohort (probable vs. unlikely) by whether or not exposure to TCDD occurred. No definition is given for that which is considered "probable" exposure to TCDD. Exposure to phenoxy herbicides does not necessarily imply exposure to TCDD in this study. The "probably exposed" category includes production workers at two plants producing PCP, 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP; dichlorprop), 4-(2,4-dichlorophenoxy)butanoic acid (2,4-DB), (4-chloro-2-methylphenoxy)acetic acid (MCPA), or 2-(4-chloro-2-methylphenoxy)-propanoic acid (MCPP; mecoprop). Those "unlikely exposed" appear to be so classified because they appeared to work in different factories. There were 181 cases of chloracne among workers in the cohort.

The results are presented below by each of the two divisions of the total cohort: a) phenoxy herbicide (ph) and/or chlorophenols and b) probable TCDD exposure. For the cohort division by ph and chlorophenols, no excess was observed for all-cause mortality, for all malignant neoplasms, for most common epithelial cancers, or for lymphomas. The four STS deaths were all in the "exposed to phenoxy herbicides and chlorophenols" subcohort (RR=2.0, CI=0.5-5.2) and all appeared 10 to 19 years after first exposure (RR=6.1, CI=1.6-15.5), with the excess risk limited to exposed sprayers (RR=8.8, CI=1.8-25.8) based on three observed deaths. None were observed in the 20 years or more category.

Increases were also noted in the exposed group for mortality from thyroid cancer (RR=3.7, CI=1.0-9.4) based on four deaths, cancer of the testis (RR=2.2, CI=0.9-4.6) based on seven deaths, other endocrine glands (RR=4.6, CI=0.9-13.5) based on three deaths, and nose and nasal cavities (RR=2.9, CI=0.6-8.5) based on three deaths. An increase in lung cancer mortality was limited to the "probably exposed" (to phenoxy herbicide and chlorophenols) group (RR=2.2, CI=1.1-4.0) based on 11 observed deaths.

The authors provided an additional analysis of STS, including five additional cases who were either alive at the end of follow-up or who had died from another cause. They concluded that the results suggest that STS in these workers "is compatible with a causal role for chlorophenoxy herbicides, though not specifically for those probably contaminated with TCDD."

The authors present only a limited analysis based on 215 and 294 expected total cancer deaths in the "probable" vs. "unlikely" exposed groups, respectively. There was a slight increase in mortality from all cancers for the probably versus the unlikely exposed groups (RR=1.1, CI=1.0-1.2 versus RR=0.9, CI=0.8-1.1), but no increase in either STS or NHL based on 4 and 11 total cases, respectively. There was also an increased mortality for testicular cancer in the group probably exposed to TCDD versus those probably not exposed (RR=3.0 vs. 1.6) based on seven total deaths and for thyroid cancer (RR=4.3 vs. 3.1) based on four total deaths. These latter two differences are not significant and, while interesting because of TCDD's known effects on these organs, add little to the information base.

While the Saracci et al. cohort is significantly larger than the other three worker cohorts (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990), the lack of both a clear definition of exposure and uniformity of exposure classification between and within plants makes the results difficult to interpret and lessens the confidence in these results. When several studies have shown a positive association of effect with the same exposure but were conducted under different circumstances, the possibility that an unknown confounder or chance produced the observed elevated effect is minimized. When different investigators working with different populations using different methods confirm an original finding, the results are more believable. TCDD tissue levels were available only from a sample of 9 of

the 181 workers with chloracne (median = 340 ng/kg, range 98 to 659 ng/kg). For 17 external controls, the median was 16 ng/kg while the range was 0 to 23.3 ng/kg. This suggests that some of the controls were exposed to TCDD. Unfortunately, no further analysis was presented on these workers.

There are several problems with this study. A portion of the Saracci et al. cohort consists of Danish workers from the Lynge (1985) study. None of them are reported by Saracci et al. (1991) as having had any exposure to 2,4,5-T. Lynge indicates that 2,4,5-T was produced at the Kemisk Vaerk Køge (KVK) facility in Denmark from 1951 until the end of 1980. However, in a later update (Lynge, 1993), she maintains that the excess is due to exposure to phenoxy herbicides other than 2,4,5-T because only 5.3 tons of 2,4,5-T were produced in 1951-1952. This statement is somewhat contradicted in her methods paper (Lynge, 1987), where she discloses that 350 tons of 2,4,5-T esters were produced during the period 1951-1981 based on purchased 2,4,5-T acid. Perhaps there occurred more exposure to 2,4,5-T than was asserted by the author. This suggests the possibility that exposure misclassification may be present in the Saracci et al. study. There may be potentially as many as 3,844 workers who had exposure to 2,4,5-T and consequently 2,3,7,8-TCDD. Many or all of them were considered as unexposed in the Saracci et al. study.

Lynge in her studies reported on five histologically confirmed cases of soft tissue sarcoma. These are listed as cases 2, 3, 4, 5, and 9 in Table IV of the Saracci et al. study. Two are considered alive for the purposes of the Saracci et al. study even though in the later 1993 Lynge study these same two are listed as deceased. The remaining three are reported by Saracci et al. as deceased. Two of these three are coded to cancer sites other than STS. Only one is correctly coded to STS. This suggests that underreporting of STS as the underlying cause of death is a problem in this study, which is consistent with the findings of Suruda (1993) that STS is underreported generally on death certificates. Added evidence of underreporting of STS is provided by the death certificate's cause of death for the two who were deceased after 1984. Both were coded to a cancer site other than STS. Altogether, four out of five of the confirmed STSs in the Lynge study were coded to causes other than STS.

7.5.4. Other Studies

Four studies containing portions of the same cohort reported above were reported elsewhere (Lynge, 1985, 1987, 1993; Coggon et al., 1986; Kogevinas et al., 1993; Bueno de Mesquita et al., 1993). Lynge (1985) reported a study of cancer incidence among persons employed in the manufacture of phenoxy herbicides in Denmark. The cohort consisted of 4,459 persons from two factories. One factory contributed 615 cohort members who had worked in the years 1951-1981. The only phenoxy acids manufactured and packaged at this plant were MCPA and mecoprop unlikely to contain TCDD. The other factory, Kemisk Vaerk Køge, contributed 3,844 cohort members who had worked in the years 1933-1981. At this plant, MCPA, 2,4-D, and lesser amounts of mecoprop, dichlorprop, and 2,4,5-T were manufactured and packaged. The investigators were unable to classify cohort members by the specific types of phenoxy herbicides to which they were exposed. However, in this plant, where exposure to TCDD-contaminated 2,4,5-T probably did occur, a significant excess risk of STS (4 observed vs. 1.00 expected, CI=1.09-10.24) was noted by the author in those workers who had achieved a minimum 10 years of latency. Unfortunately, individual tissue measurements of 2,3,7,8-TCDD were not included in this study.

In an update of the earlier study, Lynge continues to report an increase in the risk of STS with four cases reported to be in persons exposed to phenoxy herbicides (standardized incidence ratio [SIR]=2.3, CI=0.6-5.8). Just as before, this excess occurred in workers employed for more than 1 year in the Kemisk Vaerk Køge factory (SIR=6.4, CI=1.3-18.7). The author concluded that her study continues to provide evidence that exposure to phenoxy herbicides increases the risk of STS.

However, Lynge maintains that only small amounts of 2,4-D and "negligible" amounts of 2,4,5-T were produced at the KVK factory. That this amount of 2,4,5-T was negligible is somewhat at odds with data from an earlier paper in which she discussed the design of her ongoing cohort study (Lynge, 1987). In the 1987 paper, she reported that although 5.3 tons of 2,4,5-trichlorophenol were produced in 1951 and 1952, 350 tons of 2,4,5-T esters were produced from 1951-1981 based on purchased 2,4,5-T acid. This varied from zero to as much as 63 tons in any one year. Very likely, the term "negligible" is used in a relative sense—relative to the amounts of the other herbicides produced in the KVK

factory, which were produced in much larger amounts. Actual exposure to 2,4,5-T may have been greater than the impression given in Lynge's 1993 study. Most of the potential exposure was to MCPA, MCPP, 2,4-DP, and various dyes and pigments. MCPA, MCPP, 2,4-DP, and the nondioxin-containing phenoxy herbicides have not heretofore been seriously thought of as possible causes of cancer in humans.

Lynge also found that the risk of non-Hodgkin's lymphoma was not elevated in persons potentially exposed to phenoxy herbicides. She did find what she calls a "puzzling" 3.5-fold excess risk in employees of KVK employed in other manufacturing departments. No detailed information on production in these areas was included in the study.

Little additional information is provided concerning any increased risks of other forms of cancer, except for a statement that multiple myeloma and cervical cancer in women and malignant melanoma in men were significantly increased. No numbers are given for these statements. A significant excess risk of lung cancer seen in the earlier study is now borderline significant in this study (obs.=13, SIR=1.6, C.I.=0.9-2.8). The author is now arranging to have serum tissues in some of her subjects analyzed for their dioxin content.

Coggon and colleagues (1986) conducted a study of 5,754 workers at a British plant that manufactured and formulated MCPA from 1947 until 1982 and operated its own aerial and tractor-mounted spraying service from 1947 until 1972. The authors stated that other phenoxy acids were handled "at times" and that, "in comparison with MCPA, 2,4,5-T was handled only on a small scale."

Coggon et al. (1991) conducted a study of four British cohorts of manufacturers of phenoxy herbicides, including 2,4,5-T, comprising 2,239 men employed sometime during the period 1963 to 1985. All four of these cohorts were included in the Saracci et al. study previously discussed. Follow-up was to the end of 1987 through the National Health Service Central Register and the National Insurance Index. Comparisons were with the national population. Factory A produced 2,4,5-T only beginning in 1968, while the remaining three factories formulated it only beginning in 1959, 1960, and 1970, respectively. No tissue measurements were conducted on any members of the cohort. A slight excess of lung cancer was noted (19 observed, 14.2 expected). Two non-Hodgkin's lymphomas also were

observed (0.87 expected). No STSs were observed (0.18 expected). Total cancer also was not increased (37 observed, 36.85 expected).

This cohort has not been followed for a sufficiently long enough time to expect latent effects to manifest themselves. The authors assumed that the slight increase in lung cancer was probably due to cigarette smoking or a chance occurrence based on the observation that most of the lung cancer deaths occurred less than 10 years after first exposure to phenoxy compounds. Phenoxy herbicides produced or formulated at these factories include 2,4-D, MCPA, 2,4-DP, 2-methyl-4 chlorophenoxy butyric acid (MCPB), MCPP, phenoxybutyric acid (PBA), parachlorophenoxyacetic acid (PCPA), and phenoxyacetic acid (PAA). In addition, other herbicides were also made here. The author says they were exposed to a multiplicity of chemicals. Except for the slight increase in lung cancer, which is in the same direction as the findings from the earlier cohort studies, this study contributes little to the elucidation of the risk of cancer from exposure to 2,3,7,8-TCDD.

Kogevinas and her colleagues (1993) studied a group of 701 occupationally exposed women who were enrolled in IARC's International Registry of Persons Exposed to Phenoxy Herbicides and Their Contaminants. These workers were also included in the earlier Saracci et al. (1991) study. The likelihood of exposure to TCDD was based on individual job histories, company records, and company exposure questionnaires. Actual measurements of TCDD serum levels in women were not available according to the authors, so that confirmation of exposure could not be performed. Both national cancer incidence rates and national death rates were used to generate expected cases and deaths utilizing the methods of the Saracci IARC study.

The overall cancer risk did not exceed expected (SIR=96, CI=0.6-1.4) based on 29 cases. However, the group with the greatest potential for exposure to TCDD-contaminated chlorophenoxy herbicides produced a significant excess risk of cancer of all sites (SIR=222, CI=1.0-4.2) based on nine cases. The risk was observed within the first 10 years of exposure, with no elevated risk appearing after the 10th year of observation. For those women who had probable exposure to TCDD, the risk of dying from cancer was slightly elevated as well (SMR=165, CI=0.4-4.8) based on three deaths.

This study suffers from many of the same problems as the Saracci et al. (1991) study. In addition, it is a study of a small population and as such cannot be considered sensitive to the detection of small risks. These same workers were also subject to exposure to other toxic chemicals in the workplace, which may also have an effect on the risk of cancer.

However, the elevated cancer risk in women exposed to TCDD-contaminated phenoxy herbicides is consistent with a hypothesis of overall increased cancer risk seen in other studies from exposure to TCDD or TCDD-like contaminants.

Recently, another study was published (Bueno de Mesquita et al., 1993) of a cohort of 2,310 workers in two plants involved in the manufacture and preparation of phenoxy herbicides (not necessarily 2,4,5-T) in the Netherlands. These workers were also included in the IARC International Registry of Persons Exposed to Phenoxy Herbicides and Their Contaminants and hence part of the Sarraci et al. (1991) study. Some 963 were considered by the author to be exposed to phenoxy herbicides, while 1,111 were considered not exposed. The follow-up periods were somewhat skewed between the two subcohorts as well. The workers of one plant were followed from 1955 to 1985, and those at the other were followed from 1965 to 1986.

Only a slight increase occurred in total cancer mortality based on 31 deaths (SMR=107, 95% CI=73-152) utilizing The Netherlands' national rates. A slightly higher risk of total cancer was seen based on 10 deaths (SMR=137, CI=66-252) in 139 workers probably exposed to dioxins during or immediately after a 1963 industrial accident in which dioxin was released into the atmosphere.

When compared with nonexposed workers, mortality due to all cancers was insignificantly elevated (RR=1.7, 95% CI=0.9-3.4) while that due to respiratory cancer was also insignificantly elevated (RR=1.7, 95% CI=0.5-6.3). This group was too small to provide enough power to detect significant site-specific cancers.

Although the size of the cohort seems large, actually only about 549 workers in Factory A had a potential for exposure to TCDD-contaminated 2,4,5-T and the higher chlorinated dioxins. No one at Factory B was exposed to 2,4,5-T because it was not produced there. At Factory A, the SMR for lung cancer was elevated insignificantly to 165 based on 6 deaths in the 20-year latent category. No serum dioxin measurements are

available to substantiate exposure to dioxin. No soft tissue sarcomas were reported although only 0.28 were expected. However, the authors did conclude that the SMR of 73 for Factory A, the SMR of 118 for Factory B, and the SMR of 137 for the cohort exposed to the accident are not inconsistent with the possibility of a carcinogenic effect of TCDD in humans.

Wiklund and Holm (1986) studied a massive cohort of 354,620 Swedish men who were recorded as having an agriculture or forestry job according to the census of 1960 versus 1,725,845 Swedish men in all other industries. The primary exposure in those jobs was postulated to be MCPA; 2,4-D and 2,4,5-T were also used to a lesser extent. The authors found that the relative risk of STS was only 0.9. This study has several deficiencies that reduce its usefulness in determining the risk of STS due to exposure to 2,3,7,8-TCDD: (1) a lack of individualized exposure data, (2) only 15% of Swedish agricultural and forestry workers were estimated to be exposed to phenoxyacetic acids and 2% to chlorophenols, (3) Swedish agricultural workers have a decreased cancer risk and tend to use health services less frequently, (4) classifying workers according to a 1-week employment status in October of 1960 as reported in a census invites the possibility of misclassification, and (5) the crude rate of STS in agricultural and forestry workers based on data in the study is 5.45 per 100,000 person-years, and in the remaining workers it is 5.00 per 100,000 person-years. Both rates are high compared with rates from other nations (1 to 3 per 100,000 person-years).

A few years later, Wiklund et al. (1988, 1989) produced two new cohort studies that superficially appear to contradict the earlier findings of Hardell and Eriksson. Wiklund et al. followed some 20,245 licensed pesticide applicators in Sweden from date of license in 1965 or after until December 31, 1984. Some 72% were estimated to have been exposed to phenoxy herbicides 1 day or longer (based on questionnaire data sent to a random sample of 273 persons in the cohort). The relative risk for soft tissue sarcoma reported in the first study was found to be 0.9, with a mean follow-up time of 13.9 years. Even after a 10-year latency, the risk for soft tissue sarcoma was only 1.0 based on four deaths. With respect to the second study of all other cancer sites, major significant deficits were found in several sites followed for an average of 12.2 years until December 31, 1982. No report is given

concerning loss to follow-up or vital status. The authors also report that "the cohort may have been observed for too short a time to reveal any excess risk . . . ."

The authors describe a major disadvantage of these studies to be a "lack of individual exposure data" and that information is available "for only a sample of the cohort." Even the length of exposure of individual applicators to phenoxy herbicides is not available. What is presented is information that herbicide use in the 1950s was only 19%, and in the 1960s it increased to just 49%. By the 1970s, however, it was up to 67%. On the other hand, pesticide use is reported to be 92% during the same period. This seems to indicate that perhaps less than half had any exposure to the phenoxy herbicides at the time of licensing and perhaps for many years afterwards. Furthermore, information is presented about the presence or absence of chloracne, another marker of exposure to 2,3,7,8-TCDD.

In addition to missing important information regarding the vital status of this cohort by the end of the follow-up, no information is available concerning the distribution of person-years "at risk" generated by the "lost to follow-up" group. Among the cancer sites reported to have significantly reduced risks are total cancer, liver, pancreas, lung, and kidney.

Furthermore, the extent of exposure to the agent of concern, 2,3,7,8-TCDD, may not be extensive among licensed applicators in Sweden. The entire discussion is centered on exposure to "phenoxy herbicides." The authors state that the most widely used phenoxy herbicides in Sweden are MCPA, mecoprop, and dichlorprop. None of these contain 2,3,7,8-TCDD as a contaminant. 2,4-D and 2,4,5-T have also been used to a "lesser extent" according to the authors. Sweden prohibited the use of 2,4,5-T in 1977. If, as the authors state, only 19% used herbicides in the 1950s, increasing to 49% in the 1960s, it suggests that far fewer applicators were exposed to small quantities of 2,3,7,8-TCDD for a long enough period of time to produce any effects. Furthermore, only 68.2% of the cohort could have attained the age of 59 by the close of the study in 1984. This hints at the likelihood that the full impact of exposure on mortality has not yet been achieved. In fact, the authors report that the "latency time may anyhow be too short to detect increasing risks of cancer . . . ."

7.5.5. Summary

The cohorts assembled by Fingerhut et al. (1991), Manz et al. (1991), and Zober et al. (1990) are important because they contain sizable proportions of persons with substantial TCDD exposures. These exposures were documented, at least in subsets of the cohorts, by blood and/or adipose tissue measurements, workplace measurements, and the occurrence of chloracne. The Saracci et al. cohort, while significantly larger, is assembled with nonuniform exposure criteria for TCDD exposure, leading to less confidence in the results.

The exposures and methods in the three former studies in males were similar enough to warrant aggregating the results. Within each study, relative risks were estimated by summing the observed and expected numbers of deaths across categories of age, race, and calendar time, and then dividing the totals to produce relative risk estimates in the form of standardized mortality ratios. Thus, aggregate relative risks can be obtained simply by summing the observed and expected numbers of deaths across the studies. Alternatively, the aggregate relative risk could have been derived by weighting the individual relative risks from each study by the inverse of the variance. A separate analysis of the three studies using estimates of lifetime dose intake is presented in Section 8.5.

As shown in Table 7-5, the studies by Manz et al. (1991) and Zober et al. (1990) add little to the information provided by the study by Fingerhut et al. (1991), except to increase the precision of the relative risk estimates (as indicated by a narrowing of the confidence intervals). Even when aggregated, however, the estimates for cancers of connective and soft tissues, non-Hodgkin's lymphoma, and stomach cancer are highly imprecise. With this important limitation, the results suggest little or no increase in the risk of non-Hodgkin's lymphomas. They also suggest increased risk—especially among persons with relatively high exposure and relatively long latency—for connective and soft tissue cancers, for lung cancer, and for all cancers combined. Some confounding from exposure to asbestos cannot be ruled out in the Fingerhut study.

The estimates of increased risk for lung cancer and for all cancers combined are considerably more precise (Table 7-5). The elevations for these cancers also appear to be more pronounced in the subcohorts of relatively high exposure and relatively long latency

Table 7-5. Summary of Results for Selected Cancers From Follow-up Studies of Chemical Manufacturing and Processing Workers Exposed to TCDD

		Total cohorts				Subcohorts with high exposure, long latency, or both			
Cancer	Study	Observed deaths	Expected deaths	Relative risk	95% Confidence interval	Observed deaths	Expected deaths	Relative risk	95% Confidence interval
Connective and soft tissue cancers	Fingerhut (1991)	4	1.2	3.3	1.1 - 8.0	3	0.3	10.0	2.5 - 27.3
	Manz (1991)	0	0.4[a]	0.0	0.0 - 7.5	0	0.1[a]	0.0	0.0 - 30.0
	Zober (1990)	0	0.1[a]	0.0	0.0 - 30.0	0	0.0[a]	0.0	0.0 - 99.9
	Total	4	1.7	2.4	0.7 - 5.7	3	0.4	7.5	1.9 - 20.4
Non-Hodgkin's lymphomas	Fingerhut (1991)	10	7.3	1.4	0.7 - 2.4	2	2.1	1.0	0.2 - 3.1
	Manz (1991)	3	2.4[a]	1.2	0.3 - 3.4	NA	NA	NA	NA
	Zober (1990)	0	0.6[a]	0.0	0.0 - 5.0	0	0.2[a]	0.0	0.0 - 15.0
	Total	13	10.3	1.3	0.7 - 2.1	2	2.3	0.9	0.1 - 2.9
Lung cancer	Fingerhut (1991)	89	80.1	1.1	0.9 - 1.4	40	28.8	1.4	1.0 - 1.9
	Manz (1991)	30	21.3	1.4	1.0 - 2.0	NA	NA	NA	NA
	Zober (1990)	6	5.6	1.1	0.4 - 2.2	3	1.2	2.5	0.6 - 6.8
	Total	125	107.0	1.2	1.0 - 1.4	43	30.0	1.4	1.1 - 1.9
Stomach cancer	Fingerhut (1991)	10	9.7	1.0	0.5 - 1.8	4	2.9	1.4	0.4 - 3.3
	Manz (1991)	12	9.9	1.2	0.7 - 2.1	NA	NA	NA	NA
	Zober (1990)	3	2.7	1.1	0.3 - 3.0	2	0.5	4.0	0.7 - 13.2
	Total	25	22.3	1.1	0.7 - 1.6	6	3.4	1.8	0.7 - 3.7
All cancers combined	Fingerhut (1991)	265	229.9	1.2	1.0 - 1.3	114	78.0	1.5	1.2 - 1.8
	Manz (1991)	93	75.2	1.2	1.0 - 1.5	34	23.9	1.4	1.0 - 2.0
	Zober (1990)	23	19.7	1.2	0.8 - 1.7	7	4.2	1.7	0.7 - 3.3
	Total	381	324.8	1.2	1.1 - 1.3	155	106.1	1.5	1.2 - 1.7

[a]Estimated as a proportion of expected deaths from all cancers combined (see text).
NA, not available.

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than in the total cohorts. Because they come from comparisons between blue-collar workers and national populations, it is reasonable to suspect that these estimates—especially for lung cancer—are inflated to some degree by confounding due to cigarette smoking, but the limited analyses presented suggest that the association is not a chance occurrence (Table 7-2). On the other hand, the counterinfluence of the healthy worker effect, exposure misclassification, and diagnostic error would tend to force risk estimates downward. The risk estimates may be expected to be too small in this event.

The results in males are consistent with results from animal studies to some degree. In Chapter 6, it was shown that in a lifetime TCDD bioassay male rats developed lung cancer, and in an initiation-promotion study ovariectomized rats exposed to TCDD developed lung tumors, while intact rats similarly exposed did not. Furthermore, the mice in the NCI study developed fibromas and fibrosarcomas. Also, TCDD affects the immune system and has been shown to be a tumor promotor in animal liver and skin assays. Either or both of these actions could lead to increased total cancer. With respect to health effects in females, the study by Saracci et al. (1991) suggests a possible increase in breast cancer, but the results are considered preliminary in view of the small numbers and less certain exposure. On the other hand, Kogevinas et al. (1993) reported an increase in cancer incidence, all causes, among women who were exposed to chlorophenoxy herbicides contaminated with TCDD. However, no excess was observed for breast cancer. Still, in another study by Bertazzi et al. (1993) to be discussed later in the section on Seveso, Italy, a deficit of breast cancer and endometrial cancer was seen in women living in geographical areas contaminated by dioxin. TCDD exposure might be expected to result in decreased breast cancer in females, based on similar observations in rats and on TCDD's action on downregulation of the estrogen receptor. However, this is very species-, tissue-, and age-specific.

7.6. CASE-CONTROL STUDIES IN GENERAL POPULATIONS

In this section on case-control studies, the discussion will focus chiefly on the cancer sites, i.e., STS and non-Hodgkin's lymphoma, that have been suggested by the earlier Swedish studies as being associated with exposure to the phenoxy herbicides. This is a reflection of the interest shown in these two cancer sites over the last decade. Few, if any,

case-control studies have been completed on other cancer sites. And, of course, if other cancer sites are not studied, the risk of cancer cannot be evaluated. It is meaningless to do case-control studies on total cancer.

7.6.1. Sweden

Hardell, Eriksson, and colleagues conducted four studies of soft tissue sarcomas (Hardell and Sandström, 1979; Eriksson et al., 1981, 1990; Hardell and Eriksson, 1988) and one study of malignant lymphomas (Hardell et al., 1981) among men living in different parts of Sweden. In all studies, cases and their matched controls were considered exposed if they reported phenoxy acid or chlorophenol exposures lasting at least 1 day and occurring at least 5 years before the case's date of diagnosis.

The nature of the phenoxy acid exposures differed across the Swedish study locales. In northern Sweden, most exposures occurred in the use of 2,4,5-T and 2,4-D in combination in forestry applications, often by knapsack spraying (Hardell and Sandström, 1979; Hardell and Eriksson, 1988; Hardell et al., 1981; Hardell, 1981a, b). Phenoxy acid exposures not involving 2,4,5-T became progressively more common, on a proportional basis, in the central and southern regions in which agricultural herbicide uses predominated (Eriksson et al., 1981, 1990). Whereas exposures not involving 2,4,5-T made up only 22 percent of all phenoxy acid exposures in the first northern sarcoma study (Hardell and Sandström, 1979; Hardell, 1981a, b), they accounted for 27 percent in the study in central Sweden (Eriksson et al., 1990) and 58 percent in the study in southern Sweden (Eriksson et al., 1981). Exposures defined only as phenoxy acid exposures are therefore less useful as indicators of exposure to TCDD and related compounds in southern Sweden than in the central and northern parts of the country. Furthermore, none of these studies provide information dealing with how much exposure each subject may have had.

The reports contain little information on the specific chlorophenol preparations to which the cases and controls were exposed. Occasional statements in some of the manuscripts suggest that most chlorophenol exposures occurred in the sawmill and pulp industries, that they primarily involved pentachlorophenol, and that they seldom involved

trichlorophenols. Thus, most of the reported chlorophenol exposures entailed exposures to the higher-chlorinated PCDDs and PCDFs but not to TCDD.

Because exposure prevalences were generally low and because phenoxy acids and chlorophenols tend to be used in different occupations, very few persons reported joint exposures. Thus, it is efficient to control potential confounding in the analysis of data from these studies by comparing each exposure category (phenoxy acids vs. chlorophenols) with the category composed of all persons who reported no exposure to phenoxy acids or chlorophenols. Based on this method of analysis, relative risk estimates from all five studies are presented in Table 7-6, with the sarcoma studies arranged in order of publication. The measure of effect here is the "odds ratio" that is an estimate of the relative risk when the cancer is relatively rare and henceforth will be called the relative risk.

The results for 2,4,5-T are the only results pertinent to TCDD exposures only. Because of the small number of cases and controls reporting 2,4,5-T use in southern Sweden, the confidence interval for the relative risk estimate from the study in that part of the country (Eriksson et al., 1981) is extremely wide. A separate relative risk estimate for 2,4,5-T could not be computed from the data in the second northern sarcoma study (Hardell and Eriksson, 1988). The published report, however, did state that all of the cases and most of the controls exposed to phenoxy acids were exposed to preparations including 2,4,5-T (Hardell and Eriksson, 1988). The report also gave a relative risk of 3.5 for TCDD exposure, but no confidence interval or counts of cases and controls was provided.

The studies were conducted in two phases. The lymphoma study (Hardell et al., 1981), the first northern sarcoma study (Hardell and Sandström, 1979), and the southern sarcoma study (Eriksson et al., 1981) were published between 1979 and 1981. The remaining two sarcoma studies (Hardell and Eriksson, 1988; Eriksson et al., 1990) appeared about a decade later. The relative risk estimates from these more recent studies are consistently lower than those from the earlier studies (Table 7-6). Thus, systematic differences between the two sets of studies may be an explanation for the heterogeneity of results.

The first set of studies received a considerable amount of criticism concerning the methods by which the exposure information had been obtained (Hardell, 1981b; Cole, 1980).

Table 7-6. Relative Risks of Soft Tissue Sarcomas and Malignant Lymphomas in Relation to Phenoxy Acid and Chlorophenol Exposures in Five Case-Control Studies in Sweden

Exposure category and measure	Malignant lymphoma Northern Sweden 1974-1978 (Hardell et al., 1981)	Soft tissue sarcoma Northern Sweden 1970-1977 (Hardell and Sandström, 1979)	Soft tissue sarcoma Southern Sweden 1974-1978 (Eriksson et al., 1981)	Soft tissue sarcoma Northern Sweden 1978-1983 (Hardell and Eriksson, 1988)	Soft tissue sarcoma Central Sweden 1978-1986 (Eriksson et al., 1990)
Not exposed to phenoxy acids or chlorophenols					
Cases	108	33	85	41[a]	171[b]
Controls	303	187	206	255[a]	179[b]
Exposed to phenoxy acids, chlorophenols, or both					
Cases	61	19	25	13[a]	47[b]
Controls	32	19	13	56[a]	33[b]
Relative risk (95% CI)	5.3 (3.3 - 8.7)	5.7 (2.7 - 11.8)	4.7 (2.3 - 9.5)	1.4 (0.7 - 2.9)	1.5 (0.9 - 2.4)
Exposed to phenoxy acids					
Cases	41	13	14	9	23
Controls	24	14	5	22	18
Relative risk (95% CI)	4.8 (2.8 - 8.3)	5.3 (2.3 - 12.2)	6.8 (2.4 - 19.4)	2.5 (1.1 - 5.9)	1.3 (0.7 - 2.6)
Exposed to 2,4,5-T					
Cases	29	11	7	NA	19
Controls	23	10	1	NA	11
Relative risk (95% CI)	3.5 (2.0 - 6.4)	6.2 (2.5 - 15.8)	17.0 (2.1 - 140.0)	NA	1.8 (0.8 - 3.9)
Exposed to chlorophenols					
Cases	50	7	11	4	15
Controls	35	6	8	34	3
Relative risk (95% CI)	4.0 (2.5 - 6.5)	6.6 (2.1 - 20.9)	3.3 (1.3 - 8.6)	0.7 (0.2 - 2.2)	5.2 (1.5 - 18.4)

[a]Assuming no joint exposure to phenoxy acids and chlorophenols.
[b]Computed from Table 3 in the original report (Eriksson et al., 1990) with the assumption of no phenoxy acid exposures among persons with low-grade chlorophenol exposures.
NA, not available.

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The basic concern was the possibility of bias from differential exposure misclassification between cases and controls (sometimes called "observational bias" or "interviewer and recall bias"), with false-negative reports of exposure suspected as being more common among the controls and false-positive reports more common among the cases. Much of the discussion focused on telephone interviews that were conducted by research staff who were aware of the purpose of the study and of the case or control status of the respondents. These interviews were conducted with selected participants to confirm reported exposures and to resolve uncertainties on postal questionnaires, which were the primary sources of exposure information. For living cases, controls were selected from the Swedish National Population Registry. For deceased cases, controls were selected from the Swedish National Registry for Causes of Death. As the criticisms of these procedures have echoed through the years (Bond et al., 1989b; Colton, 1986), no quantitative analysis has been made of the degree of bias that would have been required to produce the very strong associations reported in the first three studies (Table 7-6). Of greater importance, analyses by Hardell based solely on the questionnaire information (Hardell, 1981b) have been largely overlooked. These analyses produced relative risk estimates very similar to those obtained when the information from the supplemental interviews was used.

Hardell also went so far as to enroll a series of colon cancer patients (Hardell, 1981) as a sort of "positive control" group. In contrast to soft tissue sarcomas and malignant lymphomas, colon cancer turned out not to be associated strongly with phenoxy acid or chlorophenol exposures. Hardell and Eriksson made a similar finding in one of the newer studies (Hardell and Eriksson, 1988) when they included a control group consisting of a variety of cancers along with a set of general population controls. The cancer controls were drawn at random from the Swedish Regional Cancer Registry.

Despite Hardell's conclusion that "the previously reported associations . . . cannot to any essential degree be explained by observational bias in the studies" (Hardell, 1981b), he and his colleagues imposed procedures designed to reduce the potential for such bias in their subsequent studies (Eriksson et al., 1990; Hardell et al., 1981). When lower relative risk estimates were produced (Table 7-6), the researchers suggested that one explanation might

have been the improved methods of exposure assessment. Again, controls were from national population registries.

The investigators suggested that another explanation for the reduced relative risk estimate for phenoxy acids and soft tissue sarcomas in the study in central Sweden (Table 7-6) "could be the decade in which exposure occurred" (Eriksson et al., 1990), with the implication that exposures were higher in earlier decades. They supported this suggestion with an analysis in which only those phenoxy acid exposures occurring in the 1950s were considered. This analysis yielded a higher relative risk estimate of 2.3 (95% CI=1.0-5.4). Unfortunately, no basis of comparison exists because analyses by calendar time of exposure were not conducted in any of the other studies.

As an alternative explanation for the lack of an elevated relative risk estimate in connection with chlorophenols in the second northern sarcoma study (Table 7-6), the authors offered "random variation" due to a low number of exposed subjects (Hardell and Eriksson, 1988). The prevalence of chlorophenol exposures among the controls in that study (10.9%) was several times *higher* than in the earlier northern sarcoma study (2.9%) (Hardell and Sandström, 1979) and virtually identical to the prevalence in the lymphoma study (10.4%) (Hardell et al., 1981). The phenoxy acid exposure prevalences were highly uniform in all three northern studies: 7.2% in the lymphoma study (Hardell et al., 1981), 6.8% in the first sarcoma study (Hardell and Sandström, 1979), and 7.1% in the second sarcoma study (Hardell and Eriksson, 1988). The chlorophenol exposure prevalence among the controls in the first northern sarcoma study (Hardell and Sandström, 1979) seems to have been low. However, the overall consistency of some excess risk is perhaps more important than actual levels of the excess.

For three of the studies by Hardell and colleagues, relative risk estimates can be computed restricting the data to persons who had worked in agriculture and the other occupational categories in which the exposures of interest tend predominantly to occur (Table 7-7). For the lymphoma study (Hardell et al., 1981) and the sarcoma study in southern Sweden (Eriksson et al., 1981), the results for exposure to phenoxy acids, chlorophenols, or both in the restricted analyses are virtually identical to those obtained with the data for all subjects (Table 7-6). For the sarcoma study in central Sweden (Eriksson et

Table 7-7. Relative Risks of Malignant Lymphoma and Soft Tissue Sarcomas in Relation to Phenoxy Acid and Chlorophenol Exposures in Three Case-Control Studies in Sweden[a]

Cancer and study locale	Exposure category	Cases	Controls	Relative risk (95% confidence interval)
Malignant lymphoma, northern Sweden (Hardell et al., 1981; Hardell, 1981)[b]	Exposed to phenoxy acids, chlorophenols, or both	51	28	1.0
	Unexposed	49	145	5.4 (3.1 - 9.5)
Soft tissue sarcoma, southern Sweden (Eriksson et al., 1981)[c]	Exposed to phenoxy acids, chlorophenols, or both	14	8	1.0
	Unexposed	17	39	4.0 (1.4 - 11.3)
Soft tissue sarcoma, central Sweden (Eriksson et al., 1990)[d]	Exposed to phenoxy acids	22	15	1.0
	Unexposed	33	51	2.3 (1.0 - 5.0)

[a]Restricted to persons who worked in the occupational categories in which these exposures predominantly occur.
[b]Analysis restricted to persons employed in agriculture, forestry, or the wood products industry.
[c]Analysis restricted to persons employed in agriculture or forestry.
[d]Analysis restricted to persons employed in agriculture, horticulture, or forestry.

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al., 1990), however, the relative risk for phenoxy acids was higher (2.3) within the special occupational categories than among all subjects.

Interestingly, Eriksson and his colleagues stated that the association with the risk of STS seemed to strengthen with exposure to the higher-chlorinated dioxin isomers. His conclusion was that not only may 2,3,7,8-TCDD be a risk factor for STS but also that other higher-chlorinated dioxins may be risk factors.

The risk calculated for exposure to 2,4,5-T in the 1950s was somewhat higher at 2.94 (95% CI=1.1-8.0). However, exposure to 2,4,5-T during the span of the study was nonsignificant at 1.8 (95% CI=0.9-3.9) excluding the chlorophenols. Exposure to dioxin-containing phenoxyacetic acids or chlorophenols, excluding nondioxin-containing herbicides, produced a significant risk estimate of 2.4 (95% CI=1.3-4.5). Exposure to high-grade pentachlorophenols produced a risk ratio of 3.9 (95% CI=1.2-12.9).

These analyses are important because many of the mechanisms by which biases might occur would be related to occupation. For instance, biases in case identification, control selection, or nonparticipation that might be related to occupational status (e.g., by its link to socioeconomic status) would not be expected to be as great in analyses conducted within the occupational categories as in analyses of the overall data. The potential for confounding by occupational exposures encountered in the same lines of work would also be reduced in the occupationally restricted analyses. Several researchers and reviewers (Johnson, 1990; Pearce et al., 1985; Blair et al., 1985) have noted reports of farmers being at increased risk of malignant lymphomas and other cancers and have mentioned a wide range of potentially responsible exposures, "including pesticides, solvents, oils and fuels, dusts, paints, welding fumes, zoonotic viruses, microbes, and fungi" (Blair et al., 1985). (Studies of farmers and other agricultural workers are not included in this review because mere membership in these occupational categories is insufficient as an indicator of exposure to such substances as 2,4,5-T or chlorophenols.)

In the original reports, occasional attempts to assess exposure-response trends produced mixed results. In general, the reported exposure periods were short in all of the studies. In the first northern sarcoma study, for instance, 93% of all reported phenoxy acid exposures lasted 1 year or less, 74% lasted 6 months or less, and 33% lasted 30 days or less

(Hardell and Sandström, 1979; Hardell, 1981b). Reported exposures in southern Sweden were even briefer, with 53% lasting 30 days or less (Eriksson et al., 1981).

Hardell et al. recently aggregated the four soft tissue sarcoma studies in a re-analysis examining exposures to herbicides contaminated with TCDD and other dioxins (Hardell et al., 1991). Increasing trends in risk with duration of exposure (<1 year and ≥ 1 year) and "latency" (5-19 years and ≥ 20 years since first exposure) were numerically impressive, being based on the totals of 434 cases and 948 controls from all the studies (Table 7-8). The problem of concomitant exposures was not solved in these analyses, however, and an analysis of the aggregated data obscured the pronounced heterogeneity of results among the individual studies (Table 7-6).

Regardless of the exposure definition, considerable heterogeneity exists among the relative risk estimates from the four soft tissue sarcoma studies (Table 7-6). (Tests of homogeneity yield two-tailed p-values of 0.002 for phenoxy acids, chlorophenols, or both; 0.02 for phenoxy acids; 0.03 for 2,4,5-T; and 0.01 for chlorophenols.) In this circumstance, aggregation of results across studies is not indicated and, instead, a search should be made for explanations for the heterogeneity.

Two additional studies of malignant lymphomas and one study of soft tissue sarcomas were conducted by independent research teams in southern Sweden. Olsson and Brandt's (1988) study consisted of 167 men diagnosed with non-Hodgkin's lymphoma in the years 1978-1981 and 140 controls from the Swedish National Population Registry. Men who reported handling phenoxy acids or chlorophenols for at least 1 day were considered exposed. However, the main focus of the study was to evaluate the contribution of organic solvent exposure to the risk of non-Hodgkin's lymphoma. Persson et al. (1989) studied 54 cases of Hodgkin's disease, 106 cases of non-Hodgkin's lymphoma, and 275 controls of both genders from the population registry of Sweden. The cases were diagnosed in the years 1964-1986, but only those who were still alive in 1986 were included. The authors did not ask specific questions about phenoxy acid use. Wingren et al. (1990) studied 96 men with soft tissue sarcomas diagnosed in the years 1975-1982, 450 general population controls, and 200 cancer controls from the regional cancer registry. Because the results did not differ substantially between the two control groups, only those obtained from analyses with the

Table 7-8. Mantel-Haenszel Odds Ratios for Soft Tissue Sarcoma Among Persons Exposed to All Dioxins, TCDD, and Dioxins Other Than TCDD in Four Case-Control Studies Involving 434 Cases and 948 Controls[a]

Substance and variable	No exposure	Exposure < 1 Yr[b]		Exposure ≥ 1 Yr	
		Latency 5 - 19 Yr	Latency ≥ 20 Yr	Latency 5 - 19 Yr	Latency ≥ 20 Yr
All dioxins					
No. of cases	352	24	34	3	21
No. of controls	865	22	52	0	9
OR	1.0	2.4		6.4	
90% CI	--	1.7 - 3.4		3.5 - 12	
TCDD					
No. of cases	352	18	22	1	5
No. of controls	865	14	25	0	2
OR	1.0	3.0		7.2	
90% CI	--	2.0 - 4.5		2.6 - 20	
Other dioxins					
No. of cases	352	6	12	2	16
No. of controls	865	8	27	0	7
OR	1.0	1.7		6.2	
90% CI	--	0.98 - 2.9		2.9 - 13	

[a]OR denotes odds ratio and CI confidence interval.
[b]All subjects were exposed for at least 1 day. Data for latency periods were combined to determine the odds ratios.
Source: Hardell et al. (1991).

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general population controls are reported here. The authors had to resort to job-associated uses, of which one was called "unspecified chemical work, potential exposure to phenoxy herbicides and chlorophenols," because only limited information could be obtained about specific chemical exposures from postal questionnaires and selected, supplemental telephone interviews.

Results from these three studies are summarized in Table 7-9. Persson et al. (1989) found strong associations, Wingren et al. (1990) found an association of intermediate strength, and Olsson and Brandt (1988) found very little association. These studies are limited by the lack of specificity in their exposure information.

7.6.2. United States

Zahm, Cantor, and colleagues from the National Cancer Institute have reported results from three case-control studies of exposure to 2,4,5-T or 2,4-T as well as other pesticides and herbicides in four Great Plains states (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992). The first study was conducted in Kansas (Hoar et al., 1986). It included soft tissue sarcomas, Hodgkin's disease, and non-Hodgkin's lymphomas, but detailed analyses were confined to the non-Hodgkin's lymphomas. The two subsequent studies, one conducted in eastern Nebraska (Zahm et al., 1990) and the other in Iowa and Minnesota (Cantor et al., 1992), evaluated non-Hodgkin's lymphomas. Cancer risks at other sites from exposure to 2,4-D calculated from these groups are the subject of later studies. These studies did not consider chlorophenol exposures, and only those persons who ever lived or worked on a farm were asked questions about pesticide exposures. Farmers and nonfarmers were asked about home and garden use of pesticides. Thus, all nonfarmers were considered unexposed. As in southern Sweden (Table 7-6), the vast majority of phenoxy acid exposures did not involve 2,4,5-T and those that did virtually always involved 2,4-D as well. The relevance of these studies to the focus on TCDD and related compounds in this review is therefore somewhat limited.

Results for 2,4,5-T from the three studies are summarized in Table 7-10. Among all subjects and among farmers, only the study in eastern Nebraska (Zahm et al., 1990) suggests an increase in risk. All three studies were conducted with virtually identical methods and no

Table 7-9. Relative Risks of Soft Tissue Sarcomas, Non-Hodgkin's Lymphomas, and Hodgkin's Disease in Relation to Phenoxy Acid and Chlorophenol Exposures in Two Case-Control Studies in Southern Sweden

Authors, study period	Cancer	Exposure	Relative risk (confidence interval)[a]
Olsson and Brandt, 1978-1981 (Olsson and Brandt, 1988)	Non-Hodgkin's lymphomas	Phenoxy acids	1.3 (0.8 - 2.1)
		Chlorophenols	1.2 (0.7 - 2.0)
Persson et al., 1964-1986 (Persson et al., 1989)	Non-Hodgkin's lymphomas	Herbicides	4.9 (1.3 - 18)
	Hodgkin's disease	Herbicides	3.8 (0.7 - 21)
Wingren et al., 1975-1982 (Wingren et al., 1990)	Soft tissue sarcomas	Unspecified chemical work, potential exposure to phenoxy herbicides or chlorophenols	1.6 (0.8 - 3.3)

[a]Confidence intervals are 95% in the Olsson and Brandt study and 90% in the other two studies.

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Table 7-10. Relative Risks of Non-Hodgkin's Lymphomas in Relation to Farm Use of 2,4,5-T in Case-Control Studies in Kansas, Eastern Nebraska, Iowa, and Minnesota

Occupational category	Exposure category and measure	Kansas (Hoar et al., 1986) Cases	Kansas (Hoar et al., 1986) Controls	Eastern Nebraska (Zahm et al., 1990) Cases	Eastern Nebraska (Zahm et al., 1990) Controls	Iowa and Minnesota (Cantor et al., 1992) Cases	Iowa and Minnesota (Cantor et al., 1992) Controls
All subjects	Exposed	3	18	13	27	25	48
	Unexposed	167	930	188	696	597	1,197
	Relative risk	0.9		1.8		1.0	
	95% confidence interval	0.3 - 3.2		0.9 - 3.5		0.6 - 1.7	
Farmers	Exposed	3	18	13	27	25	48
	Unexposed	130	644	134	512	331	650
	Relative risk	0.8		1.8		1.0	
	95% confidence interval	0.2 - 2.8		0.9 - 3.7		0.6 - 1.7	
Exposed farmers and unexposed nonfarmers	Exposed	3	18	13	27	25	48
	Unexposed	37	286	54	184	266	547
	Relative risk	1.3		1.6		1.1	
	95% confidence interval	0.4 - 4.6		0.8 - 3.4		0.6 - 1.8	

Source: Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992.

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information on herbicide application methods in any of the reports indicate any exposure conditions peculiar to eastern Nebraska.

The third set of relative risk estimates in Table 7-10 were computed using the investigators' procedure of including only the exposed farmers and the unexposed nonfarmers, with the unexposed farmers excluded. Comparing exposed farmers with unexposed nonfarmers shows that it is possible that risk estimates could be influenced by potential confounding effects that are germane to farmers, i.e., exposure to other pesticides or herbicides in large quantities. Furthermore, if the results of follow-up efforts are markedly different in farmers versus follow-up efforts in nonfarmers, then this difference also might add some uncertainty to the accuracy of risk estimates. In these analyses, the relative risk estimates from the studies in Kansas, Iowa, and Minnesota are somewhat higher and the estimate from the eastern Nebraska study is somewhat lower than in the two more conventional analyses.

Formal homogeneity tests across the three studies yield two-tailed p-values of 0.4 in the analysis of all subjects, 0.3 in the analysis restricted to farmers, and 0.6 in the third analysis. Ordinarily, especially considering the virtually identical methods used in the three studies, these results would be considered sufficient justification to compute summary estimates. Summary (maximum likelihood) estimates of relative risk are virtually identical in all three groups of subjects, with point estimates of 1.2, lower 95% confidence limits of 0.8, and upper 95% confidence limits of 1.7 to 1.8.

Woods and colleagues (1987) conducted a study of soft tissue sarcomas and non-Hodgkin's lymphomas in western Washington State. In this study, the principal method of phenoxy acid and chlorophenol exposure assessment was to place job titles, activities, and chemical preparations reported during interviews into categories of potential exposure. The categories were created "in consultation with local industrial and university representatives who had long-term experience with forestry, wood products, and agricultural industries in the Pacific Northwest" (Woods et al., 1987). No statement is given about the relative prevalence of 2,4-D and 2,4,5-T among the phenoxy acids used in this region. The authors did not present any information regarding tissue levels of TCDD in either cases or controls.

The results (Table 7-11) show no association between soft tissue sarcomas or non-Hodgkin's lymphomas and estimated potential for exposure to phenoxy acids or chlorophenols. The authors report, however, that the relative risk of non-Hodgkin's lymphomas associated with more than 15 years of potential exposure to phenoxy acids increased with time since the accumulation of that exposure. The relative estimates were 1.3 (95% CI=0.9-2.2) for exposures more than 5 years before diagnosis, 1.7 (95% CI=1.0-2.8) for exposures more than 15 years before, and 2.5 (95% CI=0.5-13.0) for exposures more than 25 years before. The authors stated that similar trends were not seen in any of the analyses of soft tissue sarcomas and phenoxy acids or of either cancer in connection with chlorophenol exposures. It is not possible with the available data from this study to conduct analyses restricted to persons who worked in forestry, agriculture, and the wood products industry, and in which the exposed persons are those who reported specific exposures to phenoxy acids or chlorophenols.

The western Washington State study reported two unique results. One consisted of elevated relative risks in connection with a history of chloracne based on personal interviews: 3.3 (95% CI=0.8-14.0) for soft tissue sarcomas and 2.1 (95% CI=0.6-7.0) for non-Hodgkin's lymphomas. However, the diagnoses were not medically confirmed and because only 1% of all cases and controls reported chloracne histories, the confidence intervals were extremely wide. The other intriguing result consisted of elevated relative risks of soft tissue sarcomas among persons with Scandinavian surnames (12% of the cases and controls). The estimates from this analysis were 2.8 (95% CI=0.5-15.6) for "high" estimated potential for phenoxy acid exposure and 7.2 (95% CI=2.1-24.7) for "high" estimated potential for exposure to chlorophenols. The authors noted that similarly elevated relative risks were not found for non-Hodgkin's lymphomas.

In a later study of the same study population, Woods and Polissar (1989) found a significant excess risk (OR=1.33, 95% CI=1.03-1.7) of non-Hodgkin's lymphoma among farmers compared with nonfarmers. When further examined to determine if 2,4-D or 2,4,5-T was responsible, both tended to be nonsignificantly decreased. However, frequency of use of these herbicides was not considered by the authors.

Table 7-11. Relative Risks of Soft Tissue Sarcomas and Non-Hodgkin's Lymphomas in Relation to Phenoxyacetic Acid and Chlorophenol Exposure in a Case-Control Study in Western Washington State, 1981-1984

Exposure measure	Soft tissue sarcomas	Non-Hodgkin's lymphomas
Estimated potential for phenoxyacetic acid exposure[a]		
Low	0.6 (0.3 - 1.1)	0.9 (0.6 - 1.3)
Medium	1.0 (0.6 - 1.7)	0.9 (0.7 - 1.3)
High	0.9 (0.4 - 1.9)	1.2 (0.8 - 1.9)
Any	0.8 (0.5 - 1.2)	1.1 (0.8 - 1.4)
Estimated potential for chlorophenol exposure[a]		
Low	0.9 (0.5 - 1.6)	1.0 (0.7 - 1.3)
Medium	0.9 (0.6 - 1.5)	0.9 (0.7 - 1.2)
High	0.9 (0.5 - 1.8)	0.9 (0.9 - 1.4)[b]
Any	1.0 (0.7 - 1.5)	1.0 (0.8 - 1.2)

[a]The reference category had no estimated potential for exposure (relative risk 1.0 by definition).
[b]Either the point estimate or the lower confidence limit appears to have been a typographical error in the original report. (On a logarithmic scale,
the point estimate should be centered between the two confidence limits.)
Source: Woods et al., 1987.

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Brown et al. (1990) conducted a population-based, case-control interview study of 578 white males with leukemia in Iowa and Minnesota matched to 1,245 controls living in those same states. The purpose of the study was to investigate potential agricultural hazards that may be related to a diagnosis of leukemia. The cases were derived from the Iowa Tumor Registry and a network of hospitals and pathology laboratories in Nebraska between March 1981 and October 1983. Areas with little farm activity were excluded from the study. There was a slight but marginally significant elevation of leukemia risk (OR=1.2, CI=1.0-1.5) in farmers versus nonfarmers. But for those who mixed, handled, or applied 2,4,5-T, the risk was slightly but nonsignificantly elevated (OR=1.3, CI=0.7-2.2).

In another case-control study of Iowa agricultural influences on multiple myeloma, Brown et al. (1993), using similar methodology as in her earlier study, matched 173 white males with multiple myeloma to 650 controls from Iowa. Although a slight nonsignificant elevated risk (OR=1.2, CI=0.8-1.7) was seen in farmers, the risk of multiple myeloma from exposure to 2,4,5-T was found to be nonsignificant (OR=0.9, CI=0.4-2.1). The same was true for numerous other herbicides, pesticides, and insecticides.

The authors concluded that there was little evidence to suggest any association of multiple myeloma with farming or pesticides. Neither of these studies on leukemia or multiple myeloma has shown that exposure to dioxin occurred. This is only presumed. No actual measurements were taken. Both of these studies could be considered hypothesis-generating studies because they involved multiple exposures to many different chemicals used in farming.

The major problem with U.S. case-control studies is that specific exposure to TCDD and related compounds is not identified or quantified, although information on the use of 2,4,5-T and 2,4-D is available in some studies. In some, only potential exposure to phenoxy herbicides is the exposure surrogate. This limits the usefulness of these studies.

7.6.3. New Zealand

Smith, Pearce, and colleagues conducted two studies of soft tissue sarcomas (Smith et al., 1982a, 1983, 1984; Smith and Pearce, 1986) and one study of non-Hodgkin's lymphomas (Pearce et al., 1986, 1987) among men in New Zealand. In these studies,

persons were first asked whether or not they "had worked in particular occupations in which there was potential for exposure to phenoxyherbicides or chlorophenols" (Smith et al., 1984). If the response was affirmative, "a series of subsidiary questions were asked to clarify the work done and the actual potential for exposure, firstly in general terms, and then in specific terms, seeking the identity of the chemicals used" (Pearce et al., 1986). The authors indicated that 2,4,5-T was widely used as a phenoxy acid herbicide in New Zealand over the years pertinent to these studies (i.e., prior to the early 1980s) (Smith et al., 1984). Thus, in these studies, the phenoxy acid exposure designation was considered a suitable indicator of exposure to 2,4,5-T and, thus, to TCDD. Typical uses of 2,4,5-T were in the spraying of gorse, blackberry, pasture, cereal, and peas. No actual measurements of 2,3,7,8-TCDD were made in these studies.

In the analyses of phenoxy acids, the authors distinguished between "potential" and "probable or definite" exposure. The latter category was created by deleting persons with only "possible" exposures from those with "potential" exposures. It is not clear whether the "probable or definite" designation included inferences from job titles, activities, and the like, or whether it was based solely on affirmative responses to specific questions about phenoxy acid exposures. For chlorophenols, only the "potential" designation was employed.

In these studies, the controls were patients diagnosed with other cancers. As opposed to a control group selected from the entire study population, a cancer control group offers less certainty about the degree to which its exposure distribution represents that of the study population, but greater certainty that bias from differential exposure misclassification through elimination of interviewer bias and recall bias is negligible. However, inclusion of cancer sites in the controls that may be associated with the exposure could potentially bias the risk estimate toward the null. In these particular studies, the cancer controls had an additional advantage in minimizing any bias that might have resulted from the inability of the researchers to include patients diagnosed at private hospitals. Private hospitals in New Zealand have only recently been contributing to the National Cancer Registry. In an interim report of the non-Hodgkin's lymphoma study, a second control group was drawn from the New Zealand electoral roll. The authors concluded that this control group "gave very similar

findings to those obtained with the main control group of other cancer patients" (Pearce et al., 1986).

Another unique feature of the New Zealand soft tissue sarcoma studies is that, like the mortality follow-up studies of chemical manufacturing and processing workers previously reviewed, they included only those cases classified to the International Classification of Diseases (World Health Organization, 1977) category 171, malignant neoplasms of the soft and connective tissues. This category, which does not include soft tissue sarcomas occurring in parenchymatous organs such as the stomach or uterus, accounted for about 60% of the soft tissue sarcoma cases in the studies in Sweden (Fingerhut et al., 1984). There is no indication from the Swedish studies, however, that the associations with phenoxy acids or chlorophenols differed between soft tissue sarcomas that would be classified in category 171 and those that would be classified in the categories for the involved organs (Hardell and Sandström, 1979; Eriksson et al., 1981; Hardell and Eriksson, 1988; Eriksson et al., 1990; Hardell, 1981).

In the New Zealand Study, investigators divided their soft tissue sarcoma research into two studies with very similar, but not identical, methods. The first study (Smith et al., 1982a, 1983, 1984) consisted of patients and controls with cancer registrations in the years 1976-1980. The second study (Smith and Pearce, 1986) extended case-finding through 1982 and was the subject of an extremely abbreviated report. The controls in the second study consisted of 315 of the 338 cancer controls from the non-Hodgkin's lymphoma study (Pearce et al., 1985) whose cancer registrations were during the period 1977-1981. (The results for the additional 23 controls, who were interviewed near the end of the non-Hodgkin's lymphoma study, evidently were unavailable at the time the analyses for the second soft tissue sarcoma study were conducted.)

The first sarcoma study (Smith et al., 1982a, 1983, 1984) reported very similar results for phenoxy acids and chlorophenols when all subjects were included in the analyses, with relative risk estimates of 1.3 for any "potential" exposure and 1.6 for exposures ("definite or probable" for phenoxy acids, "potential" for chlorophenols) lasting more than 1 day and occurring more than 5 years prior to diagnosis (Table 7-12). For phenoxy acid exposures classified by the latter definition, sufficient data were presented to permit an

Table 7-12. Relative Risks of Soft Tissue Sarcomas and Non-Hodgkin's Lymphomas in Relation to Potential Exposure to Phenoxy Acids and Chlorophenols in Case-Control Studies in New Zealand

Measure	Soft tissue sarcomas		Non-Hodgkin's lymphomas (1978-1981) (Pearce et al., 1987)
	First study (1976-1980) (Smith et al., 1983, 1984)	Second study (1981-1982) (Smith and Pearce, 1986)	
Cases	82	51	183
Controls	92	315	338
Phenoxy acids			
Any potential exposure			
Cases	21	NR	44
Controls	19	NR	72
Relative risk (95% CI)	1.3 (0.7 - 2.7)	NR	1.2 (0.8 - 1.8)
Probable or definite exposure >1 day, >5 years before cancer registration			
Cases	17	6	29
Controls	13	46	50
Relative risk (95% CI)	1.6 (0.7 - 3.5)[a]	0.8 (0.3 - 1.9)	1.1 (0.7 - 1.8)
Chlorophenols			
Any potential exposure			
Cases	8	NR	21
Controls	7	NR	27
Relative risk (95% CI)	1.3 (0.5 - 3.8)	NR	1.5 (0.8 - 2.7)
Potential exposure >1 day, >5 years before cancer registration			
Cases	8	NR	20
Controls	6	NR	27
Relative risk (95% CI)	1.6 (0.5 - 4.7)	NR	1.4 (0.8 - 2.6)

[a]Among farmers, 3.0 (1.1-8.3); controlling for farming by standardization, 1.9 (0.8-4.5).
CI, confidence interval; NR, not reported.

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analysis restricted to farmers. Thirty of the 82 cases, 13 of the 17 exposed cases, 44 of the controls, and 9 of the 13 exposed controls were farmers. Thus, the estimated relative risk is 3.0 (95% CI=1.1-8.3) among farmers. Controlling for farming by ("indirect") standardization yields an estimated relative risk of 1.9 (95% CI=0.8-4.5). Thus, as in some studies previously reviewed, accounting for the farmer/nonfarmer distinction has a material impact on the results from this study.

Very few details were presented for the second sarcoma study (Smith and Pearce, 1986). In comparison with a relative risk of 1.6 in the first study, the second study reported a relative risk of 0.8 for the principal measure of phenoxy acid exposure (Table 7-12, homogeneity-test p-value = 0.2 contrasting the two studies). The exposure prevalences in the two control groups were virtually identical (14.1% in the first study and 14.6% in the second), but the prevalences in the two case groups differed (exposure odds ratio = 2.0, 95% CI=0.7-5.4). Because of this difference, and because a relative risk estimate restricted to farmers cannot be computed with the data available from the second study, aggregation of the results would not be warranted.

The non-Hodgkin's lymphoma study (Pearce et al., 1986, 1987) reported little or no association with phenoxy acids and a somewhat stronger association with chlorophenols (Table 7-12). The latter association did not increase when the more restrictive measure of exposure was used. The various activities involving exposure to chlorophenols include the treatment of fence posts as well as treating pelts in meat works tanneries. Data that would permit an analysis restricted to farmers were not reported.

The authors continue to maintain that herbicide spraying is a full-time occupation in New Zealand and that none of the soft tissue sarcoma or malignant lymphoma cases had been commercial sprayers. Smith et al. (1984) estimated the prevalence of current and former commercial sprayers at approximately 1,500, which would be 0.17% of the male population of New Zealand in the early 1970s (Waterhouse et al., 1982). On the null hypothesis, therefore, only about 0.1 commercial sprayers would be expected among the cases in each of the two soft tissue sarcoma studies and about 0.3 commercial sprayers would be expected among the non-Hodgkin's lymphoma cases. Thus, soft tissue sarcoma risk could have been increased manyfold and non-Hodgkin's lymphoma risk could have been increased about 3-

fold before even one commercial sprayer would be expected in any of the case groups. As a consequence, the absence of commercial sprayers in any of the case groups is not strong evidence against an effect.

In a letter to the editor, Pearce (1989) produced tabular data from his earlier case-control study by duration of use and by frequency of use. Although he maintained that his data exhibited little evidence of an association with non-Hodgkin's lymphoma, a nonsignificant increase in the risk was seen in the category 10-19 days of use per year (OR=2.2, 95% CI=0.4-12.6) before dropping back to 1.1 in the category greater than 19 years.

In a study of nine selected applicators in New Zealand who had sprayed herbicides for a minimum of 180 months (and hence 2,4,5-T), Smith et al. (1992) found a high correlation between tissue levels of TCDD and months sprayed. This is analogous to Fingerhut's finding that tissue levels of TCDD correlate well with duration of employment in the herbicide manufacturing industry. TCDD serum levels ranged from 131.0 ppt in a sprayer with 31 years of spraying to a low of 3.0 ppt in a sprayer who sprayed for only 7 years. The average was 53 ppt for the nine sprayers who sprayed an average of 16 years. Actually, the mean average TCDD serum level in Fingerhut's lowest exposure group who worked less than 1 year was higher (69 ppt) than the mean average of sprayers in the Smith et al. study. Smith's conclusions were based on his analysis that brief exposures to TCDD probably do not contribute to the increased cancer risks seen in studies in other countries. Although it is an interesting inference, this conclusion may be somewhat overstated without some information regarding what the tissue levels of TCDD were in the individual cases and controls of those other studies, information that only recently is becoming available and not for all studies. Unfortunately, the hypothesis suggested by several occupational accidents, such as in Seveso, Italy, and Nitro, West Virginia, that one-time large doses of exposure to 2,3,7,8-TCDD could, in fact, lead to residual high tissue levels of serum 2,3,7,8-TCDD years later, could not be tested in the studies of Swedes. Hardell and colleagues have provided little information regarding tissue levels of TCDD, past or present, in individuals who were participants in their studies. One study by Nygren et al. (1986) is cited frequently as evidence that Swedish subjects who were involved in spraying phenoxy herbicides have

low levels of TCDD in adipose tissue samples. Thirty-one patients from the Regional Hospital in Umea were each relieved of a sample of adipose tissue for analysis of dioxin content. After "careful interviewing," it was determined that 13 of these patients had "sprayed" herbicide at some time during their past. Adipose tissue measurements indicated a mean of 2 ppt of 2,3,7,8-TCDD. The remaining 18 nonsprayers revealed a mean of 3 ppt.

However, in correspondence with C. Rappe (1987) regarding this study, it was determined that of the only three that were soft tissue sarcomas, adipose tissue measurements of 2,3,7,8-TCDD are reported to be 2, 2, and 9 ppt. The one STS with the highest 2,3,7,8-TCDD level (9 ppt) of any of the 31 subjects is stated by the authors to have had only 10 days of "knapsack spraying" some 25 to 29 years earlier. What is striking about these 13 "cases" is that the total levels of *all* chlorinated dioxins are considerably greater, i.e., from 168 ppt to as much as 936 ppt per patient, and that TCDD levels are a mere fraction of the total. The vast majority consist of the higher-chlorinated PCDDs. It is not clear that spraying herbicides is or ever was a major occupational endeavor of this group of 13 or that any of these patients was exposed to 2,3,7,8-TCDD in large quantities. Based on recent correspondence with Hardell (1993), none of the patients reported by Nygren were members of any of his case-control studies. The total PCDD levels in the three STSs ranged from 674 ppt to 792 ppt (Rappe, 1987). These were nearly all highly chlorinated. Although the cases with tissue samples came from Hardell's clinic, they did not come from any case-control studies.

This analysis provides little information on tissue levels in Swedish applicators with STS. Furthermore, there may be some differences in applicator practices between New Zealand and other countries such as Sweden. Professional applicators in New Zealand are registered with the New Zealand Agricultural Chemicals Board (Smith et al., 1982b, 1992). And although it might appear that they could be expected to receive a great deal of exposure to TCDD, more than half of the applicators show serum TCDD levels below 50 ppt (Smith et al., 1992). Considering that they were spraying 2,4,5-T for 7 to 31 years until just recently, it seems remarkable that the distribution of serum TCDD levels is as low as it is. The authors report that professional pesticide applicators in New Zealand are "perhaps the group most heavily exposed to agricultural use of 2,4,5-T in the world." In other studies,

shorter exposures to large quantities of TCDD-containing herbicides have occurred to a few personnel such as in the Ranch Hands Cohort, Seveso, Italy, and the Nitro, West Virginia, accident. In Fingerhut's study, employees with less than 1 year of exposure to phenoxyacetic acids had mean serum levels averaging 69 ppt 2,3,7,8-TCDD.

7.6.4. Italy

Vineis and colleagues conducted a case-control study of soft tissue sarcomas in three provinces in northern Italy (Vineis et al., 1986). Phenoxy acid exposure classifications were based on job information provided on interviews or questionnaires. The assessments were made by "[t]wo experts with experience in chemical aspects of agriculture." Cases and controls were classified into three categories: "certainly unexposed," "exposure could not be ruled out" (abbreviated below as "possibly exposed"), and "certainly exposed." The authors implied that phenoxy acid herbicides of all types (2,4-D, 2,4,5-T, and MCPA) were used in the area during the periods of interest, but were able to document only the use of 2,4-D and MCPA (MCPA and MCPP like 2,4-D do not contain dioxin and dibenzofuran impurities). Thus, this study may have limited relevance to an interest in TCDD exposures.

The study indicated an inverse association between possible or certain phenoxy acid exposure and soft tissue sarcoma risk among men and a positive association among women (Table 7-13). This latter association was restricted to women who were alive at the time the exposure information was collected. (In the other studies in this review, in which the results were stratified by vital status at the time of the interview, no appreciable differences were found.) As shown in Table 7-13, when the analysis is restricted to persons who had ever worked in agriculture ("farmers"), the relative risk among all women is reduced from 1.9 to 1.1. Sufficient data are not available for an analysis that is both restricted to farming women and stratified by vital status.

The authors offered overmatching by location of residence as an explanation for the lack of association among deceased subjects. It would be extraordinary for overmatching or nondifferential misclassification (the latter being the usual explanation when reduced relative risks are obtained with exposure information from proxy respondents) to be so strong as to bias a relative risk of 2.4 all the way down to 0.8.

Table 7-13. Relative Risks of Soft Tissue Sarcomas in Relation to Phenoxy Acid Exposure in Case-Control Study in Northern Italy, 1981-1983

		Men		Women	
Category	Measure	Unexposed	Possibly or certainly exposed	Unexposed	Possibly or certainly exposed
Living	Cases	21	2	16	5
	Controls	54	8	53	7
	Relative risk	1.0	0.6	1.0	2.4
	(95% confidence interval)	NA	(0.1 - 3.3)	NA	(0.7 - 8.5)
Deceased	Cases	13	1	6	4
	Controls	17	6	7	6
	Relative risk	1.0	0.2	1.0	0.8
	(95% confidence interval)	NA	(0.0 - 2.0)	NA	(0.1 - 4.1)
Total	Cases	34	3	22	9
	Controls	71	14	60	13
	Relative risk	1.0	0.4	1.0	1.9
	(95% confidence interval)	NA	(0.1 - 1.7)	NA	(0.7 - 5.0)
Total, farmers only	Cases	12	3	5	9
	Controls	12	14	8	13
	Relative risk	1.0	0.2	1.0	1.1
	(95% confidence interval)	NA	(0.0 - 0.9)	NA	(0.3 - 4.5)

NA, not applicable.
Source: Vineis et al., 1986.

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Rice is the principal agricultural crop in the study area and rice weeding was historically a predominantly female occupation. (Of 29 rice weeders in the study, all but two were women.) Rice weeding during the period 1950-1955 was manual and contact with the phenoxy herbicides was mainly through the skin.

Among all women in this study, rice weeding during the early 1950s is associated with a relative risk of 2.3 (95% CI=0.7-7.7). When the analysis is restricted to women who were farmers, however, the relative risk drops to 1.4 (95% CI=0.3-6.5).

7.6.5. Summary

From the standpoint of exposures to TCDD, the most important results from general-population case-control studies come from those studies conducted in northern Sweden (Hardell and Sandström, 1979; Hardell and Eriksson, 1988; Hardell et al., 1981), central Sweden (Eriksson et al., 1990), and New Zealand (Smith et al., 1982a, 1983, 1984; Smith and Pearce, 1986; Pearce et al., 1986, 1987). These studies were conducted in areas in which high proportions of phenoxy acid exposures involved 2,4,5-T. The exposure-assessment methods in these studies included the posing of specific questions about particular chemicals and herbicide preparations. Moreover, for all but the non-Hodgkin's lymphoma study in New Zealand (Pearce et al., 1986, 1987), available data permit analyses restricted to farmers and the other occupational categories within which the relevant exposures predominantly occur.

For soft tissue sarcomas, the Swedish studies are perhaps best represented by a relative risk of 2.3 (95% CI=1.0-5.4) for phenoxy acids among workers in agriculture, horticulture, and forestry in the study in central Sweden (Table 7-7) (Eriksson et al., 1990). This is justified by the following factors: the proportion of exposures to 2,4,5-T was high in this study, the methods of assessment were better, and there were analyses within relevant occupational categories. The authors in their current studies have redesigned their methods to accommodate readers' criticisms of their earlier studies and have made an effort to present risk estimates that are adjusted to reflect these criticisms.

The relative risk estimate of 3.0 (95% CI=1.1-8.3) for phenoxy acid exposure among farmers in the first soft tissue sarcoma study in New Zealand (Smith et al., 1982a, 1983,

1984) seems to indicate that farming may be a confounder in this study. Indirect standardization for farming produces a relative risk of 1.9 (95% CI=0.8-4.5).

For malignant lymphomas, the case-control studies provide less evidence of a positive association. The relative risk estimates from the study by Hardell and colleagues (Hardell et al., 1981) were very high, even among persons employed in the special occupational groups, but this study was conducted before the researchers had improved their data collection methods. The studies in New Zealand (Pearce et al., 1986, 1987), Kansas (Hoar et al., 1986), eastern Nebraska (Zahm et al., 1990), and Iowa and Minnesota (Cantor et al., 1992) are more consistent with a much smaller increase in risk, or no increase at all, from exposures to TCDD.

The remaining case-control studies (Eriksson et al., 1981; Olsson and Brandt, 1988; Persson et al., 1989; Wingren et al., 1990; Woods et al., 1987) offer mixed results, some suggesting increases in the risk of soft tissue sarcoma or malignant lymphoma and others suggesting little or no increase. The informativeness of each of these studies, however, is limited by one or more of the following important drawbacks: study areas in which most phenoxy acid exposures did not involve 2,4,5-T, a lack of information on specific chemicals and preparations to which cases and controls were exposed, and an inability with available data to conduct analyses restricted to farmers and the other occupational groups in which the exposures of interest primarily occur. Apparently, farming as an occupation appears to affect risk estimates based on the findings from several studies where occupation is considered and should be considered as a potential confounder.

Vineis et al. (1992) presents the hypothesis that the excess risk of non-Hodgkin's lymphoma seen among farmers exposed to phenoxy herbicides may be caused by viruses. Such viruses induce proliferation and immortalization of B-cells, followed by T-cell impairment leading to cell-mediated immunity. Increased risks of NHL have been observed in immunologically deficient individuals. Hypothetically, the same effect could be the result of exposure to TCDD as suggested in some mouse studies (see Chapter 4, Immunotoxicity).

7.7. STUDIES OF PULP AND PAPER MILL WORKERS

Table 7-14 summarizes results for cancers of interest from three follow-up studies of pulp and paper mill workers. These studies are important because of the potential for exposure to PCDDs in this line of work. The study by Robinson et al. (1986) was of 3,572 persons who had worked for at least 1 year between 1945 and 1955 at any of five mills in the states of California, Oregon, or Washington. The study by Jäppinen et al. (1987) was of 3,454 workers in the Finnish pulp and paper industry who had worked continuously for at least 1 year between 1945 and 1961. The study by Henneberger et al. (1989) was of 883 persons who had worked for at least 1 year at a mill in New Hampshire. Jäppinen et al. (1987) studied cancer incidence. The other two studies were mortality studies.

Individually and in the aggregate, these studies give little indication of appreciable increases in the risk of non-Hodgkin's lymphomas, lung cancer, or stomach cancer among pulp and paper mill workers. Overall, the rate of all cancers combined was somewhat lower than expected. None of the studies examined connective and soft tissue cancers specifically. Analyses of specific cancers by work location, duration of employment, and latency were only occasionally conducted in these studies. No consistent results were found that would alter substantially the impression given by the results for the total cohorts. These studies do not specifically mention exposure to the PCDDs and are not designed to evaluate the risk of cancer to PCDDs.

Other studies of cancer among paper and pulp mill workers have been restricted to information on deaths, using either proportional mortality ratios (Milham, 1976; Milham and Demers, 1984; Schwartz, 1988; Solet et al., 1989) or mortality odds ratios (Wingren et al., 1991) as measures of relative risk. These studies are not highly informative because they usually rely on minimal information in death records and because they are subject to an upward bias due to the "healthy-worker effect" (i.e., a tendency for employed groups to have favorable total mortality experience and causes of death other than cancers, when compared with the general population). The degree of bias in such studies varies, but it can be appreciable. For instance, in the cohort studied by Robinson et al. (1986), 915 deaths from all causes were observed and 1,150.3 were expected. If the relative risk estimates for stomach cancer and non-Hodgkin's lymphomas had been computed as proportional mortality

Table 7-14. Relative Risks for Selected Cancers from Follow-up Studies of Paper and Pulp Mill Workers

Cancer	Study	Observed deaths	Expected deaths	Relative risk	95% confidence interval
Non-Hodgkin's lymphomas	Robinson (1986)	12	8.9	1.3	0.7 - 2.3
	Jäppinen (1987)	2	3.5	0.6	0.1 - 1.9
	Henneberger (1989)	4	3.8	1.1	0.3 - 2.5
	Total	18	16.2	1.1	0.7 - 1.7
Lung cancer	Robinson (1986)	50	62.1	0.8	0.6 - 1.1
	Jäppinen (1987)	78	62.6	1.2	1.0 - 1.5
	Henneberger (1989)	25	28.0	0.9	0.6 - 1.3
	Total	153	152.7	1.0	0.8 - 1.2
Stomach cancer	Robinson (1986)	17	13.8	1.2	0.7 - 1.9
	Jäppinen (1987)	24	28.8	0.8	0.5 - 1.2
	Henneberger (1989)	5	4.2	1.2	0.4 - 2.6
	Total	46	46.8	1.0	0.7 - 1.3
All cancers combined	Robinson (1986)	160	211.5	0.8	0.6 - 0.9
	Jäppinen (1987)	196	203.8	1.0	0.8 - 1.1
	Henneberger (1989)	97	87.9	1.1	0.9 - 1.3
	Total	453	503.2	0.9	0.8 - 1.0

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ratios or mortality odds ratios, they would have been 1.5 and 1.7, respectively, instead of the values of 1.2 and 1.3 that were obtained from the authors' more valid comparisons of mortality rates (Table 7-14).

7.8. OTHER STUDIES

Studies of pesticide applicators are not informative because they contain little information on specific compounds and preparations to which individual persons were exposed and as such there is no evidence of exposure to TCDD. Studies with no information of this type include studies of licensed pesticide applicators by Wang and MacMahon (1979), Barthel (1981), Blair et al. (1983), Wiklund et al. (1987), Corrao et al. (1989), and a study of gardeners by Hansen et al. (1992). These studies contribute little or nothing to the discussion of TCDD or compounds like TCDD.

Axelson and Sundell assembled a cohort of 348 Swedish railroad workers who had applied amitrol, 2,4-D, and 2,4,5-T (Axelson and Sundell, 1974). In the most recent report (Axelson et al., 1980), 17 deaths from tumors were observed (11.85 expected, relative risk 1.43, p=.09). The relative risk estimate for lung cancer was 1.4 (three observed deaths, p=.37) and the estimate for stomach cancer was 2.2 (three observed deaths, p=.15). Again as in most studies, no actual measurements of TCDD are available from this paper. Only potential exposure to the herbicides 2,4-D and 2,4,5-T are mentioned without any effort to quantify the exposure.

Riihimäki et al. (1982, 1983) followed a cohort of 1,971 Finnish men who had applied 2,4-D and 2,4,5-T. With allowance for a 10-year latency period, 20 cancer deaths were observed (24.3 expected, RR=0.8, 95% CI=0.5-1.2). The relative risk for lung cancer was 1.1 (12 deaths observed, 95% CI=0.6-1.8). The author points out that because of limitations in the study materials, only powerful carcinogenic effects are likely to be seen.

7.8.1. Vietnam Veterans

Distributions of TCDD levels in serum and adipose tissue are typically indistinguishable between Vietnam veterans and comparison populations unless the Vietnam veterans group has been carefully defined on the basis of military records to have engaged in

activities known to have involved herbicide exposure (Centers for Disease Control Veterans Health Studies, 1988; Devine et al., 1990; Gross et al., 1984; Kahn et al., 1988; Kang et al., 1991; Pirkle et al., 1989; Schecter et al., 1989). Thus, the mere designation, "Vietnam veteran," is insufficient as an indicator of exposure to 2,4,5-T or TCDD exposure. This conclusion is also supported by Stellman and Stellman's (1986) review of military records for the purpose of developing an Agent Orange exposure index. Stellman and Stellman drew the further conclusion that "it is impossible to give any credence to any health effects study in which assignment of herbicide exposure levels to individual veterans is based solely on self-reports" (Stellman and Stellman, 1986). It is also insufficient to base an exposure index among Vietnam veterans on such crude information as military branch (Army, Marine, etc.), corps, or region of duty within Vietnam. Thus, a large number of studies of cancer experience among Vietnam veterans are uninformative from the standpoint of hypothetical effects of TCDD. These include studies by Breslin et al. (1988), the Centers for Disease Control (1987), Dalager et al. (1991), Fett et al. (1987), Greenwald et al. (1984), Kang et al. (1986), Kogan and Clapp (1988), Lawrence et al. (1985), and O'Brien et al. (1991).

One Vietnam veteran study by Kang et al. (1987) that further examined mortality in a subgroup of veterans who had ventured into the areas at the time when Agent Orange was being sprayed reported a nonsignificant odds ratio of 8.64 (CI=0.77-111.84) for soft tissue sarcoma. The number of cases was not provided. Presumably, these would be ground troops with a high likelihood of exposure.

The only study of cancer among Vietnam veterans at present with information on activities involving TCDD exposure is a small mortality study by Michalek et al. (1990) of 1,261 Air Force veterans of Operation Ranch Hand. These persons were responsible for the aerial herbicide spraying missions in Vietnam. The researchers compared the Ranch Hand group with a group of 19,101 other Air Force veterans who were mainly involved in cargo missions in Southeast Asia and who did not have herbicide exposure. A total of 12 cancer deaths were observed in the Ranch Hand cohort (17.0 expected, RR=0.7, 95% CI=0.3-1.1). Calculated death rates of all specific cancers of interest in this review were equal to or less than the rates in the comparison group, with the exception of bone, connective tissue, skin,

breast, and genitourinary organs. These numbers are too small for any meaningful comparisons.

Serum TCDD measurements were taken on 888 Ranch Hands (total). A few of the Ranch Hands, who were enlisted ground crew, exhibited tissue levels above 200 ppt of TCDD, but the median serum level was 12.4 ppt (range 0 to 618 ppm) for the entire group. The median serum level in the controls averaged 4.2 ppt (Wolfe et al., 1990). The majority of the Ranch Hands probably received little exposure. The subgroups of Ranch Hands that appear to have had the greatest exposure are nonflying enlisted personnel. The median serum 2,3,7,8-TCDD levels in 407 of them was 23.6 ppt. The next highest levels were in flying enlisted personnel with a median of 17.2 ppt. The remaining Ranch Hands exhibited levels that were not much elevated (under 10 ppt) from background (flying officers, both pilots and navigators, as well as nonflying officers). Not a great deal of cancer mortality is to be expected to date in this relatively youthful group, which has not quite reached the 20-year latency milestone. It would be of greater interest to reevaluate mortality and continue follow-up in the enlisted Ranch Hands only. They appear to be the subgroup with the greatest exposure. Further follow-up of the officers will probably not reveal any useful information that could be attributed to exposure to 2,3,7,8-TCDD. A more appropriate group in which to observe effects are members of the South Vietnamese Army who did the mainstay of the spraying around the perimeters of the military bases in Vietnam.

7.8.2. Residents of Seveso, Italy

Residents of Seveso, Italy, were exposed to 2,3,7,8-TCDD in a chemical accident in 1976. This group is important because of the high exposures, with approximately 200 cases of chloracne reported (Caramaschi et al., 1981). Children (Bertazzi et al., 1992) and adults (Bertazzi et al., 1989a, b) at the time of the accident are being studied separately. The group residing in the zone of highest estimated exposure consists of 556 adults and 306 children. The group residing in a zone of intermediate estimated exposure is larger, with 3,920 adults and 2,727 children. The group with lowest estimated exposure is larger still, with 26,227 adults and 16,604 children. The accuracy of the three estimated exposure zones has been questioned (Caramaschi et al., 1981; Merlo et al., 1986; Ratti et al., 1987; Merlo and

Puntoni, 1986), especially because the ranking does not seem to correspond to the occurrence of chloracne in the area.

There is no question that at least some of the residents of the most heavily exposed area (zone A) received massive exposure to 2,3,7,8-T (Mocarelli et al., 1991). The 1990 analysis, based on tissue specimens taken in 1976, found that the highest detected levels were recorded just after the accident. Six children at the time who subsequently developed severe chloracne had serum 2,3,7,8-T levels ranging from 12,100 ppt to 56,000 ppt. Four other persons with slightly less severe chloracne exhibited levels ranging from 828 ppt to 17,300 ppt. These were similar to those of nine other residents of zone A who did not develop chloracne whose serum 2,3,5,8-TCDD levels ranged from 1,770 to 10,400 ppt (Mocarelli et al., 1991). None of the latter group of nine were reported to be ill at the time of sampling.

Thus far, the population in zones A, B, and R around Seveso has been followed for 10 years (Bertazzi et al., 1992, 1989a, b). Ten cancer deaths, too few to support a meaningful analysis of specific cancers, have been observed among children (Bertazzi et al., 1992). Results for the cancers of interest among adults are summarized in Table 7-15 (Bertazzi et al., 1989b). No excesses of mortality from lung cancer, stomach cancer, or all cancers combined are apparent. A moderate and statistically imprecise elevation in the death rate from a subset of the cancers that make up the non-Hodgkin's lymphomas is evident in the second 5-year period of follow-up. An excess of greater relative magnitude, but even more imprecisely estimated, in mortality from cancers of connective and soft tissues appears to have occurred in the same time period. Because the exposed Seveso residents have been followed for only 10 years since the accident (Bertazzi et al., 1989a, b), highly informative results will not come until additional time has elapsed.

In a preliminary study of cancer incidence in the same Seveso population (Pesatori et al., 1992), the relative risk estimate of connective, subcutaneous soft tissue sarcoma of males living in zone R is reported to be significantly elevated at 2.81 based on 6 cases (CI=1.1-7.4). In zones A and B, none were observed but 0.4 were expected to occur in males and 0.2 in females. For females, the risk in zone R of STS is 1.43 based on two cases. Other cancer sites that are also elevated are certain hematologic neoplasms in males (lymphoreticulosarcoma) and hepatobiliary tract cancers in both males and females.

Table 7-15. Relative Risks for Selected Cancers Among Adults Exposed to TCDD in Seveso, Italy

Cancer	Gender	Calendar period			
		1976-1981 RR (95% CI)	1982-1986 RR (95% CI)	No. deaths	1976-1986 RR (95% CI)
Connective and soft tissues	Male	(NA)	2.8 (0.3 - 31.1)	2	5.4 (0.8 - 38.6)
	Female	(NA)	3.0 (0.3 - 33.0)	1	2.0 (0.2 - 19.0)
"Other" lymphatic tissue[a]	Male	(NA)	1.3 (0.4 - 4.5)	3	0.9 (0.3 - 2.9)
	Female	1.0 (0.1 - 8.6)	1.6 (0.4 - 5.7)	4	1.4 (0.5 - 4.1)
Lung cancer	Male	0.7 (0.5 - 1.0)	1.0 (0.8 - 1.4)	99	0.9 (0.7 - 1.1)
	Female	0.7 (0.3 - 1.7)	0.7 (0.3 - 1.9)	10	0.7 (0.4 - 1.3)
Stomach cancer	Male	0.8 (0.5 - 1.3)	0.9 (0.6 - 1.4)	40	0.8 (0.6 - 1.2)
	Female	0.9 (0.5 - 1.7)	1.0 (0.5 - 2.0)	22	1.0 (0.6 - 1.5)
All combined	Male	0.8 (0.7 - 1.0)	1.0 (0.8 - 1.1)	325	0.9 (0.8 - 1.0)
	Female	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.1)	176	0.8 (0.7 - 1.0)

[a]A subset of non-Hodgkin's lymphomas.
NA, not available.
Source: Bertazzi et al., 1989b.

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Bertazzi et al. (1993) refined this study to include a more complete vital status ascertainment without adding additional years of follow-up to the cancer incidence data in the contaminated areas surrounding the factory where the accident took place. Cancer occurrence ascertainment was confined chiefly to the Lombardy region of Italy (with a population of 9 million persons) because only there can be found an efficient hospital and discharge registration system, according to the authors. Lombardy hospitals routinely provide hospitalization data to the regional health department. These results can be found in Table 7-16.

Of note in this update is the information that only 14 cases of cancer were reported to have occurred in zone A. But this is not unexpected. Only 724 residents were reported to have lived there. This number was based on the assignment of addresses by the municipal vital statistics offices. But it is far too small to produce any meaningful results. However, in the more populated region B, with 4,824 residents, and region R, with 31,647 residents, several findings were noted (Table 7-16). In zone B, hepatobiliary cancer in females (RR=3.3, 95% CI=1.3-8.1), lymphoreticulosarcoma in men (RR=5.7, 95% CI=1.7-19.0), and multiple myeloma in women (RR=5.3, 95% CI=1.2-22.6) were significantly elevated.

In zone R, soft tissue sarcomas in men (RR=2.8, 95% CI=1.0-7.3) were the only site-specific cancers that were significantly elevated. Cancer of the genitourinary system was significantly depressed (RR=0.8, 95% CI=0.6-1.0). This is consistent with the animal data that suggest estrogen-induced protective effects in female rats.

The authors explain that the absence of cancer among chloracne victims is not unexpected at this time because of the relatively young age of the group and the small number of individuals affected.

Much has been made of the knowledge that these cancers have appeared within a relatively short period of time in the Seveso population for latent effects to have played any major role in the development of cancer; that is, they occurred too soon after the accident. And, indeed, it would appear that such is the case. But it should be remembered that a production plant making 2,4,5-T existed in that area many years prior to the date of the accident. It is possible that earlier exposure to dioxin from the preexisting plant could have been the initiating event for the cancers including the STSs. That would ensure enough time

Table 7-16. Relative Risks for Selected Cancers Among Adults Exposed to TCDD in Seveso, Italy, in Contaminated Areas B and R

Cancer	Males			Females		
	OBS	RR	95% CI	OBS	RR	95% CI
Region B						
All Malignancies	76	1.1	0.9 - 1.4	36	0.8	0.6 - 1.1
Trachea, Bronchus, Lung	18	1.1	0.7 - 1.8	0	---	---
Hepatobiliary	5	1.8	0.7 - 4.4	5	3.3	1.3 - 8.1
Liver	4	2.1	0.8 - 5.8	0	---	---
Hematopoietic System	8	2.1	1.0 - 4.3	6	1.9	0.8 - 4.4
Non-Hodgkin's Lymphoma	3	2.3	0.7 - 7.4	1	0.9	0.1 - 6.4
Lymphoreticulosarcoma	3	5.7	1.7 - 19.0	1	2.3	0.3 - 16.9
Hodgkin's Disease	1	1.7	0.2 - 12.8	1	2.1	0.3 - 15.7
Multiple Myeloma	2	3.2	0.8 - 13.3	2	5.3	1.2 - 22.6
Leukemia	2	1.6	0.4 - 6.5	2	1.8	0.4 - 7.3
Myeloid Leukemia	1	2.0	0.2 - 14.6	2	3.7	0.9 - 15.7
Region R						
All Malignancies	447	0.9	0.9 - 1.0	318	0.9	0.8 - 1.1
Trachea, Bronchus, Lung	96	0.8	0.7 - 1.0	16	1.5	0.8 - 2.5
Hepatobiliary	11	0.5	0.3 - 1.0	12	0.9	0.5 - 1.7
Liver	3	0.2	0.1 - 0.7	2	0.5	0.1 - 2.1
Connective & Soft	6	2.8	1.0 - 7.3	2	1.6	0.3 - 7.4
Non-Hodgkin's Lymphoma	12	1.3	0.7 - 2.5	10	1.2	0.6 - 2.3
Lymphoreticulosarcoma	4	1.1	0.4 - 3.2	6	1.7	0.7 - 4.2
Multiple Myeloma	1	0.2	0.0 - 1.6	2	0.6	0.2 - 2.8
Myeloid Leukemia	5	1.4	0.5 - 3.8	2	0.5	0.1 - 2.1
Genitourinary Organs	75	1.0	0.8 - 1.3	106	1.1	0.9 - 1.3
Breast	1	1.2	0.1 - 10.2			

Source: Bertazzi et al., 1993.

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for latent effects to manifest themselves. The relatively intense exposures received by these victims may also have contributed to a shortening of the latent period. Furthermore, hematopoietic tumors have a shorter latency than most carcinomas.

7.8.3. Rice Oil Poisonings in Taiwan and Japan Involving Compounds Structurally Related to Dioxin

This section discusses two similar incidents involving ingestion of rice cooked with oils accidently poisoned with PCBs and PCDFs. PCBs and PCDFs are structurally similar to the polychlorinated dioxins, and some of these are considered to be dioxin-like in their activity. The dioxin-like effects of these compounds are felt to be mediated through a cytosolic receptor (Poland, 1984; Poland et al., 1987; Goldstein and Safe, 1989). The dioxin-like polychlorinated biphenyl congeners, chlorinated dibenzofurans, and dioxins that have a high affinity to bind the Ah receptor induce similar effects in both animals and humans but appear to differ quantitatively in toxicity (McConnell, 1989; Ahlborg, Chapter 3, this document; Schecter, 1991). They appear to harm growth and reproduction, they may damage the immune system, and they also appear to cause cancer. These same effects have been observed in a number of different species including humans.

Two accidents involving ingestion of food contaminated with PCBs and dibenzofurans, in the Yusho (Japan) and Yu-Cheng (Taiwan) incidents, have been reported. The Yusho incident involved 1,900 people who in 1968 accidentally consumed up to 2 grams each of PCBs that had leaked into the rice oil at the facility where the rice oil was canned. The PCBs were primarily Kanechlor 400 that had been used as a heat exchange medium thousands of times. Commercial preparation Kanechlor 400 had a concentration that was 49% chlorinated. The use of this medium for exchange of heat resulted in an increase in the dibenzofuran contamination approximately 250 times. The final mixture that was actually present in the rice oil had a ratio of one molecule of dibenzofuran to every 200 molecules of PCBs.

These victims suffered many ill effects from their massive exposure that lasted only a few months. Tissue studies by the Japanese of the victims indicated that some of the PCBs and PCDFs were retained for many years after the initial exposure. The PCDFs are

eliminated at a slower rate than PCBs. Concentrations measured several years following the Yusho accident indicated that the ratio of PCDFs to PCBs remaining in the adipose tissue of the victims was about 1 to 4 (Kuratsune et al., 1975). Japanese researchers have attributed most of the noncancer toxic effects to the presence of the PCDFs although these effects are consistent with PCB exposure. These toxic effects include comedo formation, acneform eruptions, hyperpigmentation, and hyperkeratosis. In addition, ocular lesions such as swollen meibomian glands filled with yellow infarct-like material and pigmentation of the conjunctiva were seen, similar to those effects of TCDD. For further discussion of these and other effects, see Part B of Chapter 7, which addresses noncancer health effects in humans.

Kuratsune et al. (1988) reported a significantly increased risk of liver cancer in male victims (9 observed vs. 1.6 expected; SMR=559, $p<.01$) and a nonsignificantly increased risk in female victims (2 observed vs. 0.66 expected; SMR=304), as well as a significantly increased risk of lung cancer in male victims (8 observed vs. 2.45 expected; SMR=326, $p<.01$). Some 1,761 patients (887 males and 874 females) were followed from date of registration to the end of 1983, 15 years after the accident in October of 1968. Thirty-three male cancer deaths had occurred by this time versus 15.51 expected. In female victims, 8 cancer deaths had occurred while 10.55 were expected. Comparisons were with the age-, sex-, and cause-specific death rates of Japan and, separately, of Nagasaki and Fukuoka prefectures in 1970, 1975, and 1980. The author reports that the risk of liver cancer remained elevated even after the influence of latency, alcohol consumption, and liver disease had been evaluated. Kuratsune said that because there was an uneven distribution of deaths in the provinces where most of the victims lived, it was too early to draw any conclusions. Apparently, most of the liver cancers occurred in Fukuoka prefecture. A statistically significant excess mortality was still present in males of Fukuoka even when liver cancer deaths occurring less than 9 years after the accident were eliminated. The author stated, "Such a markedly uneven geographical distribution of deaths can hardly be explained by exposure to the toxic rice oil alone." However, he cautioned that his findings suggest that the poisoning might have caused liver cancer at least in male patients. He concludes, "Our findings should not be disregarded, however, because the hepatocarcinogenicity of PCBs in

animals has been well documented." Deaths from chronic liver diseases and cirrhosis are also elevated but not significantly.

An outbreak of illness similar to Yusho was reported among some 2,000 persons in the Taichung and Changhwa provinces of Taiwan in March 1979. The illness consisted of chloracne, hyperpigmentation, and meibomian gland dilatation. In October 1979, the illness was found to be the result of the ingestion of cooking oil contaminated with PCBs and PCDFs. Chen et al. (1980) reported on the blood PCB levels of 66 victims for which gas chromatograms had been prepared. Basically, blood concentration residues ranged from 11 ppb to 720 ppb in these patients. The mean value was 49 ppb; most values were under 100 ppb. In only two instances were the concentrations greater, at 120 ppb and 720 ppb. The authors reported that the higher value of 720 ppb occurred in a patient who had difficulty metabolizing and excreting PCB components. They also maintain that blood PCB levels of these patients are "much higher" than those of 72 Japanese Yusho patients (Koda and Masuda, 1975). Koda and Masuda reported the mean PCB value in Yusho patients was 5.9 ppb with a standard deviation of 4.5 ppb in 1973 and 1974. Chen et al. (1980) maintained that this difference is due to a lengthy time lapse from the exposure to PCB in Yusho patients before measurements were taken compared with a much shorter time lapse in Yu-Cheng patients. Furthermore, the patients of Yu-Cheng consumed a larger proportion of higher-chlorinated PCBs compared with those of Yusho and, as a result, the substance will be retained longer in the body, according to the authors. Ten years have now passed. Researchers should take a close look at this cohort now that the latent period has almost been achieved for liver cancer in order to confirm or deny that an excess liver cancer risk is present in these patients.

7.9. CONCLUSIONS

Of all the cancers examined in both the case-control and follow-up studies, STS provides the greater evidence of an association with TCDD relative to other sites. The original reports by Hardell, Sandstrom, and Eriksson of an association between STS and exposures involving TCDD-contaminated phenoxy herbicides have stood up to extensive criticism and a great deal of subsequent research. The degree of risk, as estimated in later

studies by Hardell's research group, does not appear to be as great as originally suggested, but an association with TCDD exposure is strengthened by tissue measurements of 2,3,7,8-TCDD in potentially exposed groups.

It is also possible that the herbicides, phenoxyacetic acid, and/or chlorophenols may exert a confounding effect. The results of the recent Lynge (1993) study suggest that TCDD-free phenoxyherbicides and/or chlorophenols may by themselves increase the risk of STS. Eriksson et al. (1990) further suggested that the higher-chlorinated dioxin isomers may also be carcinogenic.

Not every study that has looked for an association between TCDD exposure and soft tissue sarcoma risk has found one, but several studies of sound design and adequate size have done so to a greater or lesser extent. The results from the important cohort study by Fingerhut et al. (1991) of 5,000 chemical production and processing workers exposed to TCDD are corroborative, as are those from the second 5 years of follow-up of the persons exposed to TCDD in Seveso (Bertazzi et al., 1989a, b). The large IARC Registry cohort study also suggested an association between STS and phenoxy herbicide exposure, but the TCDD exposure component was less certain. The first New Zealand sarcoma study (Smith et al., 1983, 1984) also appeared to produce positive results when the analysis, presented above, was restricted to farmers to minimize bias. These results were produced by independent investigators using substantially different research methods and studying populations exposed under conditions much different from those in the studies by Hardell and colleagues, with TCDD exposure being the common link.

Moreover, no persuasive case has been made that the entirety of the association in these studies is real and not due to selection bias, differential exposure misclassification, confounding, or chance.

The evidence on malignant lymphomas in connection with TCDD exposure has not been substantiated, but recent evidence suggests an association between NHL and exposure to the herbicide 2,4-D (Zahm and Blair, 1992), which may contain dioxins other than 2,3,7,8-TCDD. The evidence from two large industrial cohort studies (Fingerhut et al., 1991; Saracci et al., 1991), and from the Seveso population suggest little if any evidence of increased risk. The limited evidence on TCDD exposure that can be extracted from the

extensive case-control studies on NHL by the National Cancer Institute (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) also does not indicate a consistent and pronounced increase in risk. It would be interesting to see results restricted to farmers from the New Zealand study of NHL. At the present time, however, the existing studies do not present even a minimally consistent picture of increased risks of malignant lymphoma among persons most probably exposed to TCDD.

The evidence for lung cancer and TCDD exposure comes from the three recent cohort follow-up studies (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990), all of which provided good TCDD exposure surrogates and some actual TCDD serum level samples. All three studies showed increased risks of borderline statistical significance of about 40% to 100% in their highly exposed groups and low risks in their less exposed groups. In addition to the above studies, the report of a significantly increased lung cancer risk in male victims of the Japanese rice oil poisoning accident (Kuratsune, 1988) is also suggestive of a TCDD-like effect (see discussion of animal carcinogenicity in Chapter 6). While confounding or synergism by tobacco smoke cannot be excluded, the limited analyses conducted suggest that smoking cannot explain the entire increase and that the association is real.

It must be remembered that the confounding influence of other occupational chemicals and presence of asbestos in the workplace could have added to the observed lung cancer cases in these studies and helped to increase the lung cancer risk calculated for these studies.

The evidence for an association with stomach cancer is less than that for lung cancer. While the high exposure/long duration cohorts in both the Fingerhut and Manz studies suggest increased risks of at least 40%, the estimates are based on too few deaths for any conclusions to be made. There are no case-control studies relating to TCDD exposure and either lung or stomach cancer because the cancers are too common with too many potential causes. Further research is needed.

While males comprise all the case-control studies and the bulk of the cohort study analyses, animal and mechanism studies suggest that males and females might respond differently to TCDD, which reduces estrogen levels in reproductive tissues and reduces estrogen-receptor binding in rat and mouse liver. These antiestrogenic effects are thought to

be responsible for decreased tumor incidences seen in the mammary gland, uterus, and pituitary of TCDD-treated female rats and may also be responsible for increased liver cancer seen in female but not male rats (see Sections 6.4.1 and 6.5.4) although in the Japanese rice oil poisonings, the reverse was the case. These female rat liver tumors may be ovary-dependent, while at the same time the ovaries appear to protect against TCDD-mediated tumor promotion in the rat lung (see Section 6.4.2). Thus, these complex mechanisms might very well affect human carcinogenicity in males and females differently. The only reported female cohort with good TCDD exposure surrogate information was that of Manz et al. (1991), which had a borderline statistically significant increase in breast cancer. While Saracci et al. (1991) did report reduced female breast and genital organ cancer mortality, this was based on few observed deaths and chlorophenoxy herbicide, rather than TCDD exposures. Bertazzi et al. (1993) reported a deficit of breast cancer and endometrial cancer in women living in geographical areas around Seveso contaminated by dioxin. Although Kogevinas et al. (1993) saw an increase in cancer incidence among female workers most likely exposed to TCDD, no increase in breast cancer was observed in her small cohort. In sum, TCDD cancer experience for women may differ from that of men but currently there are few results.

Other TCDD-related hormonal effects, including immune suppression, may result in multiorgan sensitivity and may contribute to the overall increased mortality from all malignancies combined seen in all four cohort production worker subcohorts with higher estimated TCDD exposures (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990; Saracci et al., 1991; Kogevinas et al., 1993). These increased relative risks, while not large (10% to 70%) are consistent and are either statistically significant or of borderline significance. While no one tissue site can account for this observed increase, lung cancer is also increased in four of these. Although these data suggest a hypothesis of "general carcinogenicity" consistent with a tumor promotor effect or immune suppression, it must be kept in mind that no single agent has ever been determined to cause an overall cancer response by the epidemiologic method. Although many carcinogens have been shown to increase the risk at several sites, the magnitude of the risk has differed with respect to each

site. One would not expect to see the same magnitude of increase in risk at every site affected from exposure to a carcinogen.

In conclusion, although there are uncertainties associated with the epidemiologic evidence that could have influenced risk estimates, the overall weight of evidence from the epidemiologic studies suggests that the generally increased risk of cancer is more than likely due to exposure to TCDD. The consistency of this finding in the four major cohort studies is corroborated by animal studies that show TCDD to be a multisite, multisex, and multispecies carcinogen.

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PART B: EFFECTS OTHER THAN CANCER

7.10. INTRODUCTION

Human exposure to 2,3,7,8-TCDD has been associated with noncancer effects in most systems. The majority of effects have been reported among occupationally exposed groups, such as chemical production workers, pesticide users, and individuals who handled or were exposed to materials treated with 2,3,7,8-TCDD-contaminated pesticides, and among residents of communities contaminated with tainted waste oil (Missouri, USA) and industrial effluent (Seveso, Italy).

These effects represent a complex network of responses ranging from changes in hepatic enzyme levels, which, based on current evidence, do not appear to be related to clinical disease, to observable alterations in the character and physiology of the sebaceous gland, as in chloracne (Calvert et al., 1992; Taylor, 1979). This section of Chapter 7 describes by system the noncancer effects associated with exposure to 2,3,7,8-TCDD. The characterization of the effects by system provides a context within which to compare the results of the various studies. However, it is important to recognize that the observed effects are not independent events but rather may be one outcome in a series of interrelated outcomes, some of which we may be incapable of measuring with the present technology or which we currently do not recognize as an outcome of exposure to 2,3,7,8-TCDD. A summary of the effects observed in humans is outlined in Tables 7-17 to 7-43.

The information describing human effects attributed to exposure to 2,3,7,8-TCDD-contaminated materials is derived from a wide variety of sources, including clinical assessments (case reports) of exposed individuals and analytic epidemiologic studies using case-control, cross-sectional, and cohort designs. The case reports describe the acute outcomes of exposure to 2,3,7,8-TCDD and provide the basis for hypothesis generation for controlled epidemiologic studies; however, they are not suitable for testing causal relationships between exposure and related effects (Ashe and Suskind, 1950; Suskind et al., 1953; Bauer et al., 1961; Goldman, 1972).

As described in the previous section, cohort and case-control studies have been used to investigate hypothesized increases in malignancies among the various 2,3,7,8-TCDD-

exposed populations (Fingerhut et al., 1991a, b; Manz et al., 1991; Eriksson et al., 1990). Cross-sectional studies have been conducted to evaluate the prevalence or extent of disease in living 2,3,7,8-TCDD-exposed groups (Suskind and Hertzberg, 1984; Moses et al., 1984; Lathrop et al., 1984, 1987; Roegner et al., 1991; Sweeney et al., 1989; Centers for Disease Control Vietnam Experience Study, 1988a; Webb et al., 1989). Many of the earliest studies were unable to define exposure-outcome relationships due to a variety of shortcomings, including small sample size, poor participation, short latency periods, selection of inappropriate controls, and the inability to quantify exposure to 2,3,7,8-TCDD or to identify confounding exposures. In more recent cross-sectional studies of U.S. chemical workers (Sweeney et al., 1989), U.S. Air Force Ranch Hand personnel (Roegner et al., 1991), and Missouri residents (Webb et al., 1989), serum or adipose tissue levels of 2,3,7,8-TCDD were measured to evaluate 2,3,7,8-TCDD-associated effects in exposed populations. The ability to measure tissue or serum levels of 2,3,7,8-TCDD for all or a large sample of the subjects confirmed exposure to 2,3,7,8-TCDD and permitted the investigators to test hypothesized dose-response relationships.

7.11. CROSS-SECTIONAL STUDIES: USES AND LIMITATIONS

Most of the studies that describe nonmalignant effects were designed as cross-sectional medical studies. These types of studies are useful for assessing the current status of the surviving study population; however, they are inherently limited by a number of factors, including survivor and participation biases, exposure and disease misclassification, recall bias, and interobserver variability. Survivor and participation biases may have occurred because the studies included only those who were living at the time of the study and did not or could not obtain similar information on those who died or were too ill to participate. Studies of groups exposed to agents that contribute to early deaths or cause severe illnesses may exclude the populations who were at highest risk. Exclusion of the sick and deceased whose condition was associated with 2,3,7,8-TCDD may erroneously cause the risk estimate to be closer to the null than the true risk.

Disease misclassification may be introduced in a variety of ways. Medical tests in many reviewed studies were most often performed once without follow-up. For some

disorders, multiple testing is preferred to obviate normal variations in some test parameters, e.g., immunologic tests, hormone levels. In other situations, self-reported medical histories were collected and, by design, were not or could not be confirmed by medical records. When the exposed group incorrectly reports more disease than the unexposed group, recall and reporting biases may falsely raise the risk estimate.

Exposure misclassification, particularly in the early studies, was a major limitation. In the earlier studies of production workers or community residents, exposure to 2,3,7,8-TCDD was determined only by an individual's presence (residing or working) in an area that was contaminated with 2,3,7,8-TCDD (Suskind and Hertzberg, 1984; Moses et al., 1984; Hoffman et al., 1986; Poland et al., 1971; May, 1973, 1982; Martin, 1984; Bond et al., 1983, 1989; Filippini et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986). The lack of a measurement to quantify exposure hindered the ability to confirm exposure and to assess the magnitude of an exposure-response relationship. If the misclassification is nondifferential, it tends to bias the measure of effect toward the null.

As described above, in later studies (Roegner et al., 1991; Sweeney et al., 1989; Centers for Disease Control Veterans Health Studies, 1988; Webb et al., 1989) researchers were able to confirm and quantify exposure to 2,3,7,8-TCDD in serum or adipose tissue. This breakthrough helped establish that certain previously exposed populations had 2,3,7,8-TCDD levels well above the background level of less than 20 picograms per gram of lipid (pg/g) (Patterson et al., 1989) and that the nonexposed comparison group was truly not exposed. Yet, because the occupational populations were last exposed to 2,3,7,8-TCDD-contaminated substances for as many as 40 years before being tested, levels attained at the time of exposure can only be estimated. It appears that for some populations with higher exposures, such as workers, the estimate reflects continuous exposure over an extended period. For example, despite the intervening period between last exposure to 2,3,7,8-TCDD and the determination of serum 2,3,7,8-TCDD levels in workers employed in the production of 2,4,5-TCP and 2,4,5-T (15 to 37 years after last occupational exposure), the duration of occupational exposure was highly correlated to serum levels of 2,3,7,8-TCDD obtained at the time of the study (1987-1988) (Pearson product moment correlation coefficient [r]=0.7) (Sweeney et al., 1989). These data suggest a strong relationship between length of

occupational exposure and serum 2,3,7,8-TCDD regardless of the length of the intervening period.

The deposition, metabolism, and excretion of high doses of 2,3,7,8-TCDD in the human system have not been fully described. A study by Pirkle et al. (1989) suggests that 2,3,7,8-TCDD decays by one-half in approximately 7.1 years, based on a one-compartment model and using a standard half-life equation. If this is true, exposures to trichlorophenol production workers may have been as high as 30,000 pg/g (Fingerhut et al., 1991a) and in excess of 50,000 pg/g in some residents of Seveso (Mocarelli et al., 1991). The data may be limited by the lack of complete information on the manner in which human metabolism handles 2,3,7,8-TCDD exposure.

This section of Chapter 7 is a selective review of studies that, to date, provide the most information on the relationship between nonmalignant outcomes and exposure to 2,3,7,8-TCDD-contaminated materials. Animal studies have been reviewed in other chapters of this document and will not be discussed in detail. Case reports will not be reviewed but will be used to provide support for the analytic studies. In the assessment of mortality from nonmalignant causes of death, only cohort studies in which standardized mortality ratios (SMR) or equivalent population-based risk ratios were calculated will be discussed.

Many of the reviewed studies describe a variety of outcomes that will be discussed by system throughout the text. Presented but not yet published works described by investigators from the U.S. Air Force and the National Institute for Occupational Safety and Health (NIOSH) will be included in the review. Before the results of any analysis are presented, each institution requires extensive external peer review of the findings. Other criteria for including studies in this review of noncancer end points are described in the beginning of Chapter 7.

7.12. DESCRIPTION OF PRINCIPAL STUDIES

In the following section we have provided a summary of the population description and methods of the studies that reported results for two or more systems or effects. Studies that are referenced only once will be described when cited. Results of these studies will be described in subsequent sections.

7.12.1. Occupational Studies

7.12.1.1. *U.S. Chemical Workers: West Virginia*

In March 1949, an explosion of a TCP reaction kettle in a chemical plant in Nitro, West Virginia, and the subsequent cleanup exposed approximately 450 workers to 2,3,7,8-TCDD-contaminated substances. Examination of the workers in 1949 revealed a number of acute symptoms "characterized by skin, eye and respiratory tract irritation, headache, dizziness and nausea" (Suskind and Hertzberg, 1984). The acute symptoms subsided but were followed within a week or two by "acneform eruption, severe muscle pain affecting the extremities, thorax and shoulders, fatigue, nervousness and irritability, dyspnea, complaint of decreased libido and intolerance to cold" (Suskind and Hertzberg, 1984). Thirty years later, two independent, cross-sectional medical studies were conducted to evaluate the long-term consequences of exposure to 2,3,7,8-TCDD-contaminated substances among the surviving workers (Suskind and Hertzberg, 1984; Moses et al., 1984).

In a study by Suskind and Hertzberg (1984), a group of 204 (of a total of 419) active and retired white male workers exposed between 1948 and 1969 to the 2,4,5-T production process and to the reactor release were included in a clinical examination program. The control group consisted of 163 (46% participation) current or former employees of the same plant but who had no self-reported exposure to 2,4,5-T production or maintenance of the facility. The study collected demographic and medical histories and performed clinical chemistries, urinalysis, pulmonary function tests, dermatologic examination, and conduction velocities of the sural sensory and peroneal motor. Multiple linear regression analysis was used to compare the exposed and nonexposed groups.

For participation in a separate study of workers at the Nitro plant, Moses et al. (1984) invited all workers documented in union records to have worked in 2,4,5-T production and a systematic random sample of workers with no known 2,4,5-T production exposure. Fifty-five percent (N=226) of the persons invited participated in the study. Lifetime occupational and medical histories were collected and clinical chemistries, urinalysis, and dermatologic examinations were conducted. Exposure to 2,3,7,8-TCDD could not be discerned due to irreconcilable inconsistencies in self-reported work histories and the lack of good company records to estimate and confirm the likelihood of exposure. Although the authors recognized

that absence of chloracne did not preclude exposure, nevertheless, they compared the results of the group with chloracne with those without chloracne. Thus, the study design was revised to explore the differences in health status in individuals with and without chloracne. Because exposed workers may have been included in the group diagnosed without chloracne, the usefulness of the data to quantify exposure-disease relationships is limited.

Two studies of this cohort also examined cancer and noncancer mortality of subsets of workers from this plant (Zack and Suskind, 1980; Collins et al., 1993).

7.12.1.2. *U.S. Chemical Workers: The NIOSH Study*

The study conducted by the National Institute for Occupational Safety and Health is a cross-sectional medical study of living workers who were previously employed for at least one day in one of two plants located in Newark, New Jersey, and Verona, Missouri. From 1951 to 1969, 490 workers employed at the New Jersey plant produced sodium 2,4,5-trichlorophenate (NaTCP), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), and 2,4-dichlorophenoxy acetic acid (2,4-D). A high proportion of chloracne and other dermatologic abnormalities and cases of porphyria and hypomania were reported among the workers at the New Jersey facility (Poland et al., 1971; Bleiberg et al., 1964), which produced some of the most heavily 2,3,7,8-TCDD-contaminated NaTCP and 2,4,5-T among production facilities whose products were surveyed (Fee et al., 1975). At the Missouri plant, NaTCP and 2,4,5-T were produced intermittently for 4 months in 1968, and NaTCP and hexachlorophene were produced continuously for 22 months between April 1970 and January 1972. Prior to the NIOSH cross-sectional study, the health of the 96 Missouri production workers had not been previously studied.

For comparison, unexposed neighborhood referents were recruited using a random sampling procedure described by Sweeney et al. (1989). Referents were selected if they reported no prior history of occupational exposure to 2,3,7,8-TCDD and matched the worker by age (within 5 years), race, and gender. A total of 586 workers were eligible for inclusion in the study, of which 400 (68.3%) were living, 142 (24.2%) were deceased, and 44 (7.5%) could not be located. All 400 living workers were invited to participate in the study; 281 (70%) were examined. A description of the study population is included in the results.

Worker and referent health and exposure status was assessed in 1987-1988 through an interviewer-administered medical and occupational history and comprehensive physical and psychological examinations (Sweeney et al., 1989). A lifetime medical history was obtained from each examined participant by interviewers who were blind to the exposure status of the respondent. Results of the pulmonary, hepatic, gastrointestinal, porphyria, mood dysfunction, and neurologic examinations have been published or accepted for publication (Calvert et al., 1991, 1992, 1993; Sweeney et al., 1993). Findings for diabetes and serum glucose level, reproductive endocrine function, and p450 metabolism have been presented in public meetings (Sweeney et al., 1992; Egeland et al., 1994; Halperin et al., 1992).

As a surrogate for cumulative exposure, serum 2,3,7,8-TCDD levels were measured in 237 workers and a random sample of 79 referents. Procedures for sample collection, preparation, adjustment for lipids, and statistical analysis were described in earlier reports (Fingerhut et al., 1989; Patterson et al., 1986a; Sweeney et al., 1990). The mean lipid-adjusted serum 2,3,7,8-TCDD level for workers was 220 pg/g, median 80 pg/g, ranging to 3,400 pg/g. The mean level was statistically significantly greater than that for referents (7 pg/g) ($p < 0.001$). Analyses of other congeners of dioxins and dibenzofurans were also conducted; only the 2,3,7,8-TCDD levels were different in the two exposure groups (Piacitelli et al., 1992).

7.12.1.3. *BASF Accident Cohort*

"On 17 November 1953, an uncontrolled decomposition reaction occurred during the production of 2,4,5-trichlorophenol at a BASF AG facility in Ludwigshafen, Germany" (Ott et al., 1993a). The reactor contents, which contained 2,3,7,8-TCDD, contaminated the building in which the TCP autoclave was housed. A series of studies have documented the effects and mortality experience of the workers present at the time of the decomposition reaction and exposed during the initial cleanup and equipment maintenance (May 1954 medical department list) (Cohort C1, N=69), individuals present during subsequent clean-demolition activities between 1954 and 1969 (Cohort C2, N=84), and a mixed group of workers identified as of December 1987 through interviews that include individuals who worked in the laboratory as safety inspectors and others who participated in the 1968-1969

demolition activities (Cohort C3, N=101) (Zober et al., 1990; Ott et al., 1993b). Two hundred forty-seven study subjects were included in a mortality study that found a significantly elevated standardized mortality ratio (SMR) for all malignant neoplasms among workers with chloracne and 20 or more years since first exposure to 2,3,7,8-TCDD-contaminated chemicals (Zober et al., 1990).

Among 79% of the living subjects, lipid-adjusted serum 2,3,7,8-TCDD levels were measured in 138 (54%) of 254 study subjects during the period 1988-1992 (Ott et al., 1993a). The geometric mean of the 2,3,7,8-TCDD levels in the entire group was 15.4 ppt (ranging from <1 to 553.0 ppt) (Ott et al., 1993a) or 43 pg/g of lipid (M.G. Ott, personal communication, 1993). Geometric means for the cohorts are as follows: C1=1,009.5 pg/g lipid; C2=48.8 pg/g of lipid; and C3=83.7 pg/g of lipid. Background levels were determined in separate analyses of 102 unexposed individuals from Germany (Päpke et al., 1992). The geometric mean for 2,3,7,8-TCDD of the external referent group was 3.0, ranging from 0.6 to 9.1 pg/g of lipid. Based on regression analyses, serum 2,3,7,8-TCDD levels were highly correlated to duration of exposure and location of exposure. Chloracne severity was positively and significantly related to 2,3,7,8-TCDD concentrations.

Comprehensive batteries of clinical chemistry measurements were also measured for the 138 subjects between 1988 and 1993 (Ott et al., 1993b). Referents were selected from among BASF employees between the ages of 50 and 69 who participated in routine occupational medical examinations from 1989 to 1991. For some tests, there were as many as 6,000 referent values. For the immunologic parameters, the referent values were obtained from a group of 42 unexposed BASF employees who participated in a separate study that examined the immunologic function of 21 extruder personnel exposed to 2,3,7,8-tetrabrominated dibenzo-dioxins (2,3,7,8-TBDD) and -furans (2,3,7,8-TBDF) (Zober et al., 1992).

7.12.2. Studies of Community Residents

7.12.2.1. *The Missouri Experience*

During 1971, 2,3,7,8-TCDD-contaminated stillbottoms were removed from a hexachlorophene production facility and mixed with waste oil. This mixture was deposited

on 45 residential, recreational, and industrial sites in southeastern Missouri in 1971 and 1972 (Daryl Roberts, personal communication). Waste oil mists were commonly used in the summer for dust control on roadways, horse arenas, truck depots, and other unpaved surfaces. Estimated contamination of the areas ranges from 1 to 2,200 ppb (Hoffman and Stehr-Green, 1989). A listing of potentially exposed persons was created (volunteers), and a survey was conducted to obtain baseline information to identify persons at high risk of exposure. Beginning in 1984, a series of studies were conducted to evaluate potential effects (Hoffman et al., 1986; Evans et al., 1988; Webb et al., 1989), including reproductive events, of residential exposure to 2,3,7,8-TCDD (Stockbauer et al., 1988).

The study by Hoffman et al. (1986) was conducted on 154 individuals (74% of total eligible) who were residents of the Quail Run Mobile Home Park between 1971 and 1983 because soil concentrations around the site were 2,200 ppb 2,3,7,8-TCDD. The comparison group of 155 (77% of total eligible) individuals was recruited from residents of a nearby mobile home park. The examination included tests for delayed hypersensitivity (the multitest Cornell Medical Index [CMI]; Merieux Institute, Miami, FL) and neurobehavioral effects, blood chemistries, urinalysis, height, weight, vital signs, and examination of the skin, peripheral pulses, lymph nodes, abdomen, and peripheral nervous system. The results of this study were plagued by the exclusion of skin test results of 150 of 294 participants due to high reader error. Furthermore, information on subject exposure to 2,3,7,8-TCDD was limited because it was based on a minimum residence of 6 months in areas with contaminated soil. Actual contact with contaminated soil was not assessed.

In the follow-up study by Evans et al. (1988), 50 persons from the initial study who did not respond to the delayed hypersensitivity skin tests were retested. These subjects were thought to have impaired immune function. The multitest CMI was reapplied to all test subjects.

Webb and colleagues (1989) examined 41 of 51 persons with various histories of exposure to 2,3,7,8-TCDD (residential, recreational, and occupational exposure) and for whom adipose tissue levels of 2,3,7,8-TCDD were measured. Of the 41 participants, 16 had adipose 2,3,7,8-TCDD levels less than 20 pg/g (within background range), 13 had levels between $\geq$20 and 60 pg/g, and 12 subjects had levels above 60 pg/g. Standard medical

examinations were conducted, and complete blood count with differential, a panel of automated chemistry tests, serum immunoglobulins, tests for porphyrins, and the multitest CMI were performed.

7.12.2.2. *Seveso, Italy*

In 1976, an explosion of a trichlorophenol reactor in a 2,4,5-T production facility in Medina, Italy, caused the contamination by 2,3,7,8-TCDD of the neighboring city of Seveso, Italy. The contaminated area was subdivided into three zones (A, B, and R) of decreasing mean soil levels of 2,3,7,8-TCDD (Mocarelli et al., 1988). The mean 2,3,7,8-TCDD concentration in Zone A was 230 $\mu g/m^2$; in Zone B, 3.0 $\mu g/m^2$; and in Zone R, 0.9 $\mu g/m^2$.

In 1979, Pocchiari et al. (1979) reported on initial efforts to screen residents in Zones A, B, and R for 2,3,7,8-TCDD-related effects. Since then, a series of cross-sectional medical studies have reported the final results of the screening (Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). Within 1 year of the reactor release, 193 cases of chloracne were identified among residents of Zones A, B, and R, most of which resolved with time (Assennato et al., 1989). For Seveso residents, a standard diagnosis of chloracne was developed, in which all cases were stratified by severity: 0, no lesions; 1, a few comedones (up to 10, minimum stage); 2, numerous comedones and cysts (light stage); 3, comedones and cysts in specific regions (medium stage); and 4, comedones and cysts spreading from the face to other regions of the body (serious stage) (Caramaschi et al., 1981). Four studies investigated possible biochemical changes, particularly liver enzyme induction and lipid levels, among the 170 children diagnosed with chloracne and control groups (Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989).

In addition to chloracne, several other studies also evaluated peripheral neuropathy. In an early report by Pocchiari et al. (1979), tests for presence of peripheral nerve dysfunction were conducted for Seveso residents and for workers at the Icmesa production facility. Assennato et al. (1989) and Filippini et al. (1981) assessed the prevalence of peripheral neuropathy, comparing residents with and without chloracne (Assennato et al.,

1989) or comparing residents having chloracne or abnormal serum hepatic enzyme levels with residents with no manifestations of 2,3,7,8-TCDD exposure (Filippini et al., 1981).

Two mortality studies, one of children ages 1-19 years and another of adults 20 years and older, examined death rates in residents of Zones A, B, and R 10 years after the explosion (1976-1986) (Bertazzi et al., 1989, 1992). The comparison population was composed of approximately 100,000 inhabitants of uncontaminated areas surrounding Seveso. Follow-up for both the young and older cohorts was 99%.

To date, the general limitation of the studies conducted in Seveso residents is the classification of exposure of subjects by residence in Zones A, B, or R, which is based on mean soil concentration of 2,3,7,8-TCDD. Although serum levels of some individuals exposed in Zone A at the time of the reactor release were in excess of 10,000 pg/g, levels of residents living in the other areas were not measured (Mocarelli et al., 1991). The weaknesses of using soil 2,3,7,8-TCDD levels to classify extent of exposure to 2,3,7,8-TCDD were aptly described by Bertazzi et al. (1989): "This (use of soil contamination) is a rather poor surrogate of exposure, and by no means an indicator of intake, since it does not take into consideration all the possible sources and ignores interindividual variability." This is the same problem encountered by some researchers investigating 2,3,7,8-TCDD-related effects among Missouri residents. One might also expand this limitation to each of the studies where environmental rather than personal levels of 2,3,7,8-TCDD contamination were used for exposure classification.

7.12.3. Studies of Vietnam Veterans

7.12.3.1. *The Vietnam Experience Study*

The Vietnam Experience Study is described by its authors as a "multidimensional assessment of the health of Vietnam veterans" (Centers for Disease Control Vietnam Experience Study, 1988a, b, c, d). This study was designed to examine effects among men who served in Vietnam.

The study population was composed of a random sample of men who enlisted in the U.S. Army from 1965 through 1971, whose military occupational status was other than "duty soldier," who enlisted for a single term with a minimum of 16 weeks active duty and who

were discharged at pay grades of E-1 to E-5. The controls were selected from among veterans enlisting during the same period but whose duty station was the United States, Germany, or Korea. Participation involved completion of a telephone survey of current and past health status by 7,924 veterans who served in Vietnam and 7,364 veterans who served outside of Vietnam. A random subsample of 2,940 Vietnam and 1,972 non-Vietnam veterans participated in the health evaluation component.

In a separate study, serum 2,3,7,8-TCDD levels were measured in a subset of the examined population: 646 Vietnam veterans who served in Vietnam during 1967 and 1968 and 97 non-Vietnam veterans (Centers for Disease Control Veterans Health Studies, 1988). The mean serum 2,3,7,8-TCDD level was not different between Vietnam (mean = 4.1 pg/g lipid [standard deviation (SD) $\pm$ 2.3]) and non-Vietnam (4.2 pg/g, SD$\pm$2.6) veterans. Two Vietnam veterans had levels above the background level of 20 pg/g: 25 pg/g and 45 pg/g.

The overall strengths of this study are that it is a large study, with good power to detect many common disorders; participation in the questionnaire part of the study was good (87% for Vietnam veterans; 84% for non-Vietnam veterans); there was good comparability between the two cohorts in demographic characteristics, although there were differential participation rates in the examination; and validation of selected self-reported effects was conducted. This study is limited primarily by the differential participation rates in the examination (75% for Vietnam veterans; 63% for non-Vietnam veterans). The low level of 2,3,7,8-TCDD in the sample of veterans made it impossible to conduct dose-response analyses.

7.12.3.2. *U.S. Air Force Ranch Hand Study*

One of the largest epidemiologic studies of U.S. military personnel stationed in Vietnam is being conducted by the U.S. Air Force. The study population consists of Air Force personnel who served in Operation Ranch Hand units in Vietnam from 1962 to 1971 and who were employed in the dissemination of Agent Orange through aerial spraying. Comparisons included Air Force personnel who flew or maintained C-130 aircraft in Southeast Asia during the same time period.

The study design includes a series of cross-sectional medical studies conducted at 5-year intervals beginning with the baseline study in 1982 (N=1,045 exposed, 1,224 unexposed). Two follow-up evaluations were conducted in 1985 (N=1,016 exposed, 1,293 unexposed) and 1987 (N=995 exposed, 1,299 unexposed). Each cross-sectional study included comprehensive physical and psychological evaluations. In the 1982 baseline and 1985 and 1987 follow-up studies, exposure was based on the comparison of the Ranch Hand group versus the comparison group. An additional analysis approximated exposure (low, medium, high) for the Ranch Hand group by using historical military data and herbicide procurement and usage records. The results of these analyses were prepared by Lathrop and colleagues (1984 and 1987). In 1988, serum 2,3,7,8-TCDD levels were measured for a sample of the 1987 Ranch Hand group (N=866) and the 1987 comparison group (N=804). The 1987 examination data were then reanalyzed using lipid-adjusted serum 2,3,7,8-TCDD levels as the relative measure of exposure. The median serum 2,3,7,8-TCDD level adjusted for lipids for the Ranch Hand group was 12.8 pg/g, ranging to 618 pg/g. For the comparison group, the median level was 4.2, ranging to 54.8 pg/g (Roegner et al., 1991).

The overall strengths of this study are that it is a large study, with good power to detect many common disorders; follow-up was very good as is continued participation of Ranch Hand and comparison populations. The physical and psychological examinations are extensive, planned to evaluate most, if not all, outcomes hypothetically associated with 2,3,7,8-TCDD. Continued reevaluation of the subjects (every 5 years) permits investigators to monitor the development of chronic diseases and to test for additional outcomes as new biochemical and toxicologic data become available. Finally, the determination of serum 2,3,7,8-TCDD levels permitted validation of the exposure matrix based on historical records and the subsequent development of disease-specific dose-response models. Repeated measures of serum 2,3,7,8-TCDD over time will also provide valuable information on its half-life in humans.

Noteworthy caveats in the study include the fact that the majority of the population currently has serum levels under the background level of 20 pg/g (median = 12.8 pg/g, range to 600 pg/g). These data suggest that, although there are some Ranch Hands who were exposed to very high levels of 2,3,7,8-TCDD, most of the study group had lower

exposures. In addition, the serum 2,3,7,8-TCDD levels indicated that the exposure matrix used in the analysis of the baseline and 1984 studies did not appropriately describe the potential for exposure. Therefore, data described in this chapter will refer only to the baseline and 1984 results where Ranch Hands as a group were compared to the comparisons and, most often, to the reanalysis of the 1987 data using serum levels.

Many analyses were performed for this study. For ease of discussion in this report, only the adjusted odds ratios for the three categories of serum 2,3,7,8-TCDD selected by Roegner and colleagues (1991) are presented and discussed. The categories are: ≤ 10 pg/g 2,3,7,8-TCDD; 15-≤ 33.3 pg/g 2,3,7,8-TCDD; and >33.3 pg/g 2,3,7,8-TCDD.

A consequence of conducting a comprehensive study in which a large number of statistical tests are performed is an increased possibility of spurious findings. The reader should be cognizant of this limitation, looking for consistencies with the results of other studies and in the toxicologic literature rather than statistical significance.

7.13. REVIEW OF EFFECTS ASSOCIATED WITH EXPOSURE TO 2,3,7,8-TCDD

The following section contains a review of the case reports and epidemiologic studies that describe effects associated with exposure to materials contaminated with 2,3,7,8-TCDD.

7.13.1. Dermal Effects

7.13.1.1. *Chloracne*

The most widely recognized dermal effect of exposure to 2,3,7,8-TCDD-contaminated substances is chloracne. Chloracne is a persistent acneiform condition characterized by comedones, keratin cysts, and inflamed papules with hyperpigmentation and a unique anatomic distribution, occurring subsequent to acute and chronic exposure to a variety of chlorinated aromatic compounds (Crow et al., 1978; Moses and Prioleau, 1985). This acne-like condition is reported to have occurred with and without other effects in at least a few workers after all reported accidents at TCP production facilities (Ashe and Suskind, 1950; Suskind et al., 1953; Goldman, 1972; May, 1973; Zober at al., 1990), among individuals involved in daily production of 2,3,7,8-TCDD-contaminated products (Bleiberg et al., 1964; Poland et al., 1971; Pazderova-Vejlupkova et al., 1981; Moses et al.,

1984; Moses and Prioleau, 1985; Suskind and Hertzberg, 1984; Bond et al., 1989), among three laboratory workers exposed to pure 2,3,7,8-TCDD (Oliver, 1975), and among at least 193 (0.6%) Seveso residents, mostly children (Reggiani, 1978; Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). Chloracne was not found among Missouri residents (Hoffman et al., 1986; Webb et al., 1989) examined 10 years after exposure or among Ranch Hand personnel (Lathrop et al., 1984; Roegner et al., 1991). In U.S. Army Vietnam veterans, chloracne-like skin lesions were rarely observed on examination (0.9% in Vietnam veterans versus 0.8% in non-Vietnam veterans, OR=1.4, 95% CI=0.7-2.9) (Centers for Disease Control Vietnam Experience Study, 1988a).

Based on reports from Seveso and studies of chemical workers, chloracne appeared shortly after exposure to 2,3,7,8-TCDD-contaminated chemicals (Caramaschi et al., 1981; Zober et al., 1990). The eruption of blackheads, usually accompanied by cysts, was observed between 2 weeks and 2 months after the reactor release (Reggiani, 1980). In Seveso, within 6 months of the explosion, 34 cases of chloracne were identified among children, whereupon a more intensive search was undertaken among school children (Caramaschi et al., 1981). In chemical workers involved in the TCP reactor release at BASF Ludwigshafen, Germany, most cases of chloracne (that were also diagnosed with cancer) developed within 2 days after first exposure (Ott et al., 1993a; Zober et al., 1990). One case of chloracne did not develop for 2 years, but the authors suggest that the etiology of this case is unclear.

For many affected individuals, the condition disappeared after discontinuation of exposure (Assennato et al., 1989) despite high serum 2,3,7,8-TCDD levels (Mocarelli et al., 1991). But for a few, the chloracne remained for many years (Suskind and Hertzberg, 1984; Moses and Prioleau, 1985). Of the 204 exposed workers in the study by Suskind and Hertzberg (1984), 52% had persistent chloracne for at least 10 years after the TCP and 2,4,5-T processes ceased, 34% reported a history of chloracne, and 14% reported no history of chloracne. Moses et al. (1984) reported that the mean duration for persistent chloracne was 26.1 ± 5.9 years.

There are very few human data from which to determine definitively the threshold level of 2,3,7,8-TCDD at which chloracne occurs or who is at greatest risk to develop

chloracne. Data from analyses of chloracne cases among chemical workers and its relationship to serum and adipose tissue levels of 2,3,7,8-TCDD and hexachlorinated (HxCDD) dioxins provide some basic yet useful information on the characteristics of chloracne cases, particularly the interindividual susceptibility to chloracne (Bond et al., 1989; Ott et al., 1987; Beck et al., 1989; Mocarelli et al., 1991). Bond et al. (1989) described 325 cases (15%) of chloracne among 2,192 workers exposed to 2,3,7,8-TCDD and HxCDDs or octachlorinated (OCDD) dioxins between 1938 and 1982 during chemical production activities (not as a result of a reactor accident). Cases were identified through company-maintained medical records; age- and calendar year-specific incidence rates were estimated based on age- and calendar year-specific person-years of employment contributed by the cohort; and risk factors were adjusted for by logistic regression. The analysis found that risk of chloracne was highest among workers who were exposed at younger ages, among those who had the longest length of exposure to 2,4,5-trichlorophenol or pentachlorophenol production operations, and among jobs rated at the highest intensity of exposure (Ott et al., 1987). These characteristics of chloracne cases in Michigan workers are consistent with those observed in chloracne cases from the BASF accident cohort (Ott et al., 1993a). Although this study may underestimate the incidence rate of chloracne due to possible underreporting or misdiagnosis of cases, and misclassification of exposure may have occurred, this study was the first of its kind to explore analytically the risk factors associated with occupationally acquired chloracne. It is unfortunate that these exposure estimates have not been validated by serum or adipose tissue 2,3,7,8-TCDD, HxCDD, and OCDD levels.

Serum levels of 2,3,7,8-TCDD and HxCDD have been measured in chloracne cases of Seveso residents (Mocarelli et al., 1991) and German chemical workers (Beck et al., 1989; Ott et al., 1993a). Mocarelli et al. (1991) described chloracne in persons from Zone A who had very high serum 2,3,7,8-TCDD levels ranging from 820 to 56,000 pg/g measured within 1 year of the reactor release (Table 7-17). The study also included other individuals from Zone A, but without chloracne, who had serum 2,3,7,8-TCDD levels that ranged from 1,770 to 10,400 pg/g. With the exception of one person with chloracne who was 16 years old at the time of the accident, all of the cases were in children under age 11. Those without chloracne, for the most part, were over age 30. It is not clear whether the

Table 7-17. Serum 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Levels in Seveso Residents with Chloracne and Adipose Tissue Levels of 2,3,7,8-TCDD and Hexachlorinated (HxCDD) Dioxins in German Chemical Workers

Author	Population	2,3,7,8-TCDD level (pg/g)[a]	HxCDD level (pg/g)[a]	Year of chloracne diagnosis	Half-life extrapolated 2,3,7,8-TCDD[b]	Half-life extrapolated HxCDD[b]
Beck et al., 1989	Chemical workers[c]	174	247	1955	750	10,000
		99	166	1955	4,010	6,720
		147	5,101	1963	2,350	81,620
		61	172	1957(?)	2,470	6,970
		50	517	1969	380	3,940
		16	58	1955	650	2,360
		1,280	1,019	1978	3,380	2,690
		49	3,442	1974(?)	210	14,760
		50	9,613	1972(?)	260	50,740
		2,252	3,087	1984	2,850	3,910
		158	1,191	1977(?)	460	3,490
		6	283	1970	40	1,970
Mocarelli et al., 1991	Seveso residents	56,000[d]	---	1976-1977		
		27,800	---	1976-1977		
		26,400	---	1976-1977		
		15,900	---	1976-1977		
		12,100	---	1976-1977		
		17,300	---	1976-1977		
		7,420	---	1976-1977		
		1,690	---	1976-1977		
		828	---	1976-1977		

[a]pg/g of lipid (parts per trillion).

[b]Half-life extrapolation calculated by authors (Beck et al., 1989) using the formula $C_o = C_{t_n} \times 2^n$ where C_o = original concentration of 2,3,7,8-TCDD or HxCDD, C_{t_n} = concentration at time t, n = number of half-life periods, and t = half-life period of 5.8 years. Exposures occurred between 1949 and 1986.

[c]Measured in 1984.

[d]Measured in 1976.

children were more susceptible to the chloranegenic effects or whether they had greater exposure to 2,3,7,8-TCDD-contaminated soil or airborne effluent.

As documented by others, adult TCP production workers also developed chloracne (Beck et al., 1989; Suskind and Hertzberg, 1984; Bond et al., 1989). Adipose tissue levels of 2,3,7,8-TCDD and HxCDD measured in adult chloracne cases of German chemical production workers suggest that these cases may have been a function of the combined exposure, making it difficult to isolate the contribution of the different chlorinated compounds. All cases had estimated adipose levels of greater than 200 pg/g 2,3,7,8-TCDD and in excess of 2,000 pg/g lipid HxCDD at the time of diagnosis. Estimated levels were based on the half-life extrapolation of the adipose tissue level measured in 1986 to the date of last occupational exposure, which may have occurred between 1949 and 1984. Similarly, Ott (1993a) found that 80% of the severe chloracne cases had estimated (back-calculated) levels of 250 pg/g. Yet, 26% of nonchloracne cases had estimated 2,3,7,8-TCDD concentrations of 250 pg/g.

Data from studies of Seveso residents conducted from 1982 to 1985 indicate that, despite high serum 2,3,7,8-TCDD levels, the chloracne resolved in all but one person by 1983 (Assennato et al., 1989). The fact that the cases of chloracne in Seveso residents resolved within 10 years may explain why no chloracne was observed in the Ranch Hand group although some serum 2,3,7,8-TCDD levels exceeded 600 pg/g in 1988 (Roegner et al., 1991) and may have been as high as 2,400 pg/g at the time of last occupational exposure, assuming 21 years since last exposure and a 7-year half-life. Nevertheless, residual chloracne was observed 30 years after first exposure among workers from Nitro, West Virginia, which may suggest that chronic high exposure to 2,3,7,8-TCDD or exposures higher than experienced by the Ranch Hands may account for long-term persistence of chloracne.

7.13.1.2. *Dermatologic Disorders Other Than Chloracne*

Dermal effects other than chloracne attributed to 2,3,7,8-TCDD exposure include a variety of symptoms and conditions that occurred less frequently than chloracne but appeared in several groups subsequent to acute and continuous exposure to 2,3,7,8-TCDD-

contaminated TCP and 2,4,5-T. Two reports indicated that after acute episodes of exposure, e.g., accidents, individuals complained of red and irritated eyes, conjunctivitis, and blepharitis (inflammation of the eyelids) (Ashe and Suskind, 1950; Baader and Bauer, 1951). Other investigators also found cases of eyelid cysts several months after acute exposure (Suskind et al., 1953; Kimmig and Schulz, 1957a, b; Poland et al., 1971; Reggiani, 1980) and up to 25 years after exposure (Moses et al., 1984; Suskind and Hertzberg, 1984).

Hyperpigmentation and hirsutism (also known as hypertrichosis or abnormal distribution of hair) were diagnosed among chemical workers in the United States (West Virginia and New Jersey) (Ashe and Suskind, 1950; Suskind et al., 1953; Bleiberg et al., 1964; Poland et al., 1971), Germany (Bauer et al., 1961; Goldman, 1972), and Czechoslovakia (Jirasek et al., 1974) who were exposed to 2,3,7,8-TCDD-contaminated TCP during manufacturing processes or industrial accidents and among laboratory workers in England exposed while synthesizing pure 2,3,7,8-TCDD (Oliver, 1975). Upon reexamination 25 years later, hypertrichosis was observed in exposed workers (5.4% exposed vs. 1.8% unexposed) from the West Virginia plant, particularly among workers with persistent chloracne on clinical examination (10.3% with persistent chloracne vs. 0% with history of chloracne only) ($p<0.001$) in one of two independent studies (Suskind and Hertzberg, 1984). A second study by Moses et al. (1984) found no evidence of hypertrichosis, although 31% of the exposed workers had evidence of residual chloracne (Moses et al., 1984). Studies of Vietnam veterans have reported no significant increase in the prevalence of either hyperpigmentation or hypertrichosis (Roegner et al., 1991; Centers for Disease Control Vietnam Experience Study, 1988a). Three cases of hypertrichosis but not hyperpigmentation were observed among Missouri residents, one with serum levels less than 20 pg/g and two with levels between 20 and 60 pg/g (Webb et al., 1989). Neither disorder was noted on examination among residents of the Quail Run Mobile Home Park (Hoffman et al., 1986).

Actinic or solar elastosis was found to be more prevalent among West Virginia workers diagnosed with active chloracne at the time of their examinations in 1979 (Suskind and Hertzberg, 1984) (exposed = 59.1% vs. unexposed = 30.1%, $p<0.01$). No significant difference was observed in the age-adjusted prevalence of actinic elastosis in workers with or

without chloracne in the study by Moses et al. (1984). Actinic elastosis is known to be directly related to sun exposure; however, the amount of sun exposure, skin type, or other factors contributing to the sensitivity of the skin to sunlight were not assessed in the report. No other studies of TCP production workers, the Ranch Hands, or U.S. Army Vietnam veterans have found an increase in the prevalence of actinic elastosis.

Among the group of workers studied by Suskind and Hertzberg (1984), three cases of Peyronie's disease were noted. Peyronie's disease is a rare condition characterized by progressive scarring of the penile membrane. No explanation for this finding was expressed nor has the condition been noted (or perhaps looked for) in other studies (Bond et al., 1989; Moses et al., 1984; Roegner et al., 1991).

In 1984, a statistically significant excess of nonmelanotic skin cancer was reported among Ranch Hand personnel involved in the aerial spraying of herbicides over Vietnam compared with a matched comparison group (Lathrop et al., 1984). The comparison group was composed of Air Force personnel assigned to cargo missions outside the sprayed areas of Vietnam. A follow-up study of the same cohorts in 1987 confirmed the excess of basal cell carcinoma and attributed the increase to sunlight exposure (Lathrop et al., 1987). However, in the reanalysis of the 1987 examination data, skin neoplasms of any kind were not related to serum 2,3,7,8-TCDD level (Roegner et al., 1991).

7.13.1.3. *Comments*

From an epidemiologic perspective, chloracne is a common consequence of exposure to chemicals contaminated with 2,3,7,8-TCDD and some other polyhalogenated hydrocarbons. Available data on serum or adipose tissue levels of 2,3,7,8-TCDD have not determined the threshold at which a case of chloracne occurs. Evidence from Bond et al. (1989) suggests that for chemical production workers the risk of chloracne may be related to the part of the process in which the workers were engaged, the amount of time spent in the contaminated region, and the intensity of the exposure while in the area. Chloracne is also related to short-term high-intensity exposures as observed in Seveso residents (Reggiani, 1980) and occupational cohorts (Ott et al., 1993a). Few studies have been successful in evaluating the relationship between history of chloracne and long-term nonmalignant effects.

However, Zober et al. (1990) noted that the SMR for all malignant neoplasms for workers with chloracne and who had at least 20 years of latency (time since first exposure) was statistically significantly elevated (SMR 201; 90% CI=122, 135). Future studies that are able to evaluate the association between history of chloracne and effects would provide useful information in this regard.

Other conditions, such as hyperpigmentation and hypertrichosis, may be more acute effects of 2,3,7,8-TCDD exposure that resolve over time, because they were not observed in studies where the cohorts were examined years after the cessation of exposure. Actinic keratosis, Peyronie's disease, and basal cell carcinoma may not be due to 2,3,7,8-TCDD because actinic keratosis and Peyronie's disease have been observed in a single cohort. Likewise, the excess basal cell carcinoma was noted only in one study group, and the results could not be replicated when a better indicator of personal exposure to 2,3,7,8-TCDD, serum 2,3,7,8-TCDD, was used as a surrogate for exposure in the statistical models.

7.13.2. Gastrointestinal Effects

7.13.2.1. *Hepatic Effects*

Changes in liver function and structure after exposure to 2,3,7,8-TCDD are commonly observed in experimental animals (Greig et al., 1973; Vos et al., 1974; Jones and Greig, 1975; McConnell et al., 1978a, b; Jones et al., 1981; Zinkl et al., 1973; Kociba et al., 1976, 1978; Gasiewicz et al., 1980; DeCaprio et al., 1986). The changes are not always consistent from one species to another, but they have prompted examination of hepatic effects among exposed human populations. As with animals, there is wide variation in the type and degree of hepatic effects reported in humans after exposure to 2,3,7,8-TCDD-contaminated materials. This section describes selected hepatic effects associated with 2,3,7,8-TCDD exposure observed in humans, including hepatomegaly and hepatic enzyme changes.

7.13.2.2. *Liver Size*

Increased liver size is consistently reported in treated animals after exposure to 2,3,7,8-TCDD (Vos et al., 1974; Allen et al., 1977; McConnell et al., 1978a, b; Kociba et al., 1978; Gasiewicz et al., 1980). Among exposed human populations, four case reports in

three populations, but not controlled epidemiologic studies, described evidence of enlarged livers or hepatomegaly. Liver size was reportedly increased among two TCP production workers in West Virginia within a few months after a TCP reactor explosion (Ashe and Suskind, 1950; Suskind et al., 1953) and among "several" production workers in Czechoslovakia exposed to TCP, the butyl ester of 2,4,5-T, and sodium pentachlorophenol (Jirasek et al., 1974). Temporary liver enlargement was observed in 5 of 22 Seveso residents who had severe chloracne (Reggiani, 1980). The hepatomegaly lasted "several" months without concomitant elevation in hepatic enzymes. Fortunately, the effect appeared to be transient. Cross-sectional medical studies of TCP production workers (Bond et al., 1983; Suskind and Hertzberg, 1984; Moses et al., 1984; Calvert et al., 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991), and Missouri residents (Webb, 1989; Hoffman et al., 1986) have found little evidence of excess hepatomegaly in the exposed populations. Additionally, no dose-response relationship was observed between serum levels of 2,3,7,8-TCDD and physical findings of an enlarged liver for either the study of Ranch Hands ($\leq$10 pg/g 2,3,7,8-TCDD, RR=0.39, 95% CI=0.11-1.33; 15-$\leq$33.3 pg/g 2,3,7,8-TCDD, RR=1.47, 95% CI=0.57-3.79; >33.3 pg/g 2,3,7,8-TCDD, RR=1.6.9, 95% CI=0.60-4.75) (Roegner et al., 1991) or the NIOSH study of TCP productions workers (two workers; four referents; OR=0.46, 95% CI=0.09, 2.43) (Calvert et al., 1992).

One Missouri resident was found to have hepatomegaly, but he also was suffering from diabetes mellitus. His adipose tissue 2,3,7,8-TCDD level was 430 pg/g (Webb et al., 1989). The differences in findings between the case reports and the controlled epidemiologic studies suggest that hepatomegaly may be a resolvable, acute effect as a result of exposure to high levels of 2,3,7,8-TCDD.

7.13.2.3. *Enzyme Levels*

Laboratory studies have demonstrated changes in hepatic enzyme levels after 2,3,7,8-TCDD exposure, although there is considerable interspecies variation in the observed effect (Zinkl et al., 1973; Kociba et al., 1976, 1978; Gasiewicz et al., 1980; Olson et al., 1980). Epidemiologic studies and case reports describe elevated liver enzymes among exposed TCP

production workers and among Seveso residents (Mocarelli et al., 1986; May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992; Ott et al., 1993b).

7.13.2.4. *GGT*

Increased levels of gamma glutamyl transferase (GGT) may suggest activity such as cholestasis, liver regeneration, or drug or xenobiotic metabolism (Table 7-18). The studies of Seveso children demonstrate an increase in GGT levels occurring shortly after the explosion and then a gradual decline to near normal levels within 5 years. In one of the earliest studies of Seveso children with (N=141) and without chloracne (N=138), 2.8% of the children with chloracne had out-of-range GGT levels, but none of the children without chloracne had an out-of-range level ($p<0.001$) (Caramaschi et al., 1981). These results were echoed in a study of children from Zones A, B, and R of Seveso, in which enzyme levels were measured yearly between June 1977 and June 1982 (Mocarelli et al., 1986). GGT levels were elevated in children of Zone A, particularly in boys, during the first 2 years after the explosion (1977: exposed = 9.73 U/L; unexposed = 7.28 U/L; $p<0.01$; 1978: exposed = 9.88 U/L; unexposed = 8.26 U/L; $p<0.05$) (Mocarelli et al., 1986). Levels in girls during the same years were elevated but did not achieve statistical significance. For the next 4 years of the study, GGT levels remained elevated in boys and girls from Zone A compared to unexposed children, but the values declined with time.

GGT was found to be elevated among TCP production workers from one plant in Great Britain and in workers from West Virginia, Missouri, and New Jersey up to 30 years after last occupational exposure to 2,3,7,8-TCDD-contaminated chemicals (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992). The findings of two studies of British workers were similar. Mean GGT levels were increased, but not statistically elevated, in workers with chloracne compared to unexposed controls tested 10 years after exposure to 2,3,7,8-TCDD-contaminated chemicals as a result of a TCP reactor explosion (chloracne, GGT=39 U/L; controls, 27.7 U/L [May, 1982]) (chloracne, GGT=32 U/L; controls, 32 U/L [Martin, 1984]). Similarly, a statistically significant excess in the proportion of individuals with abnormally high GGT levels was found among West Virginia workers with chloracne who were examined as many as 30 years after exposure (Moses et

Table 7-18. Mean Serum Levels of Gamma Glutamyl Transferase (GGT) Among Seveso and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean level[a]	(SD)	N	Mean level[a]	(SD)
	Seveso residents						
Mocarelli et al., 1986	(1977) (Boys)	52[b]	9.73	(4.72-20.04)	42[b]	7.28	(3.71-14.26[c])
	(Girls)	32[b]	9.10	(3.62-22.85)	43[b]	8.05	(2.99-21.68)
	(1982) (Boys)	106[b]	9.70	(4.29-21.93)	138[b]	8.99	(4.45-18.20)
	(Girls)	117[b]	9.04	(4.25-19.23)	140[b]	8.59	(3.85-19.14)
Assennato et al., 1989	1976	193[d]	11.94	(16.80)	---[e]	---	---
	1982	152[d]	11.67	(7.94)	123[f]	11.23	(7.41)
	1984	142[d]	10.53	(7.07)	196[f]	11.19	(7.05)
	1985	141[d]	10.57	(7.38)	167[f]	10.94	(4.68)
	Missouri residents						
Webb et al., 1989	<20[g]	16	24.0	(15.5)	---	---	---
	20-60	13	17.7	(7.23)	---	---	---
	>60	12	32.8	(23.90)	---	---	---
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	140	30.0[h]	(88.3)	141	20.1	(23.4)
May, 1982	TCP production workers in Great Britain	41	39.0	---	31	27.7[c]	---
Martin, 1984	TCP production workers in Great Britain	41	40.0	(14-91)	120	32.0	(11-90)
Moses et al., 1984	TCP and 2,4,5-T production workers in West Virginia	22[d]	26.3[c]	(27.0)	9[f]	17.4	(11.0)
Calvert et al., 1992	TCP and 2,4,5-T production workers in Missouri and New Jersey	280	58.5[c,i]	(73.7)	259	47.4	(41.1)

[a]Units = U/L.
[b]Number of samples.
[c]$p < 0.05$.
[d]Chloracne.
[e]No data for controls in 1976.
[f]No chloracne.
[g]Adipose tissue level of 2,3,7,8-TCDD in pg/g of lipid.
[h]% Abnormal: exposed = 3.6; unexposed = 3.6.
[i]% Abnormal: exposed = 10.7; unexposed = 5.0; OR=227 (95% CI=1.17, 4.39).

Table 7-18. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a]	(SD)	N	Mean level[a]	(SD)
Ott et al., 1993b	BASF accident cohort	133	30.5	(58.4)	6,708	29.9	(43.5)
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army ground troops	2,490	43.2[j]		1,972	41.1[k]	---
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[l,m]	338	31.49	---	777	34.64	---
	Low 15-≤33.3[l,m]	191	38.28	---			
	High >33.3[l,m]	182	40.82	---			

[a]Units = U/L.
[j]Geometric mean.
[k]% Abnormal (Vietnam veterans, 5.5%; non-Vietnam veterans, 4.4; OR=1.3, 95% CI=1.0-1.8).
[l] Serum 2,3,7,8-TCDD level in pg/g of lipid.
[m]Contrasted to unexposed comparison population.

al., 1984) (chloracne, mean GGT=26.3 U/L, 23% abnormal; no chloracne, mean GGT=17.4 U/L, 9% abnormal; $p<0.003$). Yet, compared to controls, GGT was not elevated in another study of West Virginia workers (Suskind and Hertzberg, 1984).

In a study by Calvert et al. (1992), the mean GGT level and the proportion of workers with out-of-range levels were statistically significantly elevated among TCP workers from New Jersey and Missouri (workers, mean GGT=58.5 U/L; unexposed referents, mean GGT=47.4 U/L, $p<0.03$; workers, 11% abnormal; referents, 5% abnormal, OR=2.27, 95% CI=1.17-4.39). Based on the logistic regression model in Table 7-19, the increases in GGT were limited to workers with high serum 2,3,7,8-TCDD levels (>100 pg/g) and extremely high lifetime alcohol consumption (>30 alcohol years) (alcohol year = 1 alcoholic beverage/day for 1 year). The contribution of other potentially confounding exposures that may have affected GGT levels was not explored in this study. Other studies of TCP production workers in Michigan and West Virginia or the BASF accident cohort did not report elevations in GGT levels (Bond et al., 1983; Suskind and Hertzberg, 1984; Ott et al., 1993b).

Both the Vietnam Experience Study and the U.S. Air Force Ranch Hand Study found statistically significant elevations in GGT levels (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991). In Army Vietnam veterans, mean GGT levels were 43.2 U/L compared with 41.1 U/L in non-Vietnam veterans (OR for out-of-range value = 1.3, 95% CI=1.0-1.8) (Centers for Disease Control Vietnam Experience Study, 1988a). In the 1987 follow-up study, the comparison of the adjusted mean GGT level in the comparison group and in each of the three Ranch Hand groups defined by 2,3,7,8-TCDD level found statistically significant increases in the Ranch Hand population ($\leq$10 pg/g 2,3,7,8-TCDD, $p<0.017$; 15-$\leq$33.3 pg/g 2,3,7,8-TCDD, $p<0.043$; >33.3 pg/g 2,3,7,8-TCDD, $p<0.001$) (Roegner et al., 1991).

7.13.2.5. *AST and ALT*

Abnormal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may indicate liver cell damage from a number of causes, including hepatic necrosis, metastatic carcinoma, or obstructive jaundice (AST and ALT) or infectious or toxic hepatitis

Table 7-19. Logistic Regression Model for an Out-of-Range Serum Gamma-Glutamyltransferase[a] (GGT) Level Using the Categorical TCDD[b] Exposure Measure[c]

Variable	Beta	Standard error of the estimate	X^2	p
Intercept	-4.12	0.50	67.01	<0.001
Exposure (worker = 1, referent = 0)	0.37	0.43	0.74	0.195
Per alcohol-year	-2.4×10^{-6}	2.6×10^{-3}	0.00	0.999
Current alcohol drinker (yes = 1, no = 0)[d]	0.90	0.42	4.70	0.030
Alcohol-years/exposure interaction[e,f]	7.2×10^{-3}	3.5×10^{-3}	4.24	0.039
Triglyceride level	3.8×10^{-3}	1.1×10^{-3}	11.94	<0.001

[a]Reference value: 96 IU/L; a level was considered out of range if it exceeded the reference value. Reference values were defined as the 95th percentile for the referent cohort.
[b]TCDD = 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.
[c]N=536 observations.
[d]The results from this logistic regression analysis change little when this term is dropped from the model.
[e]Interaction between alcohol-years and exposure.
[f]Exposure odds ratios (ORs) for an abnormal γ-glutamyltransferase level among workers by selected alcohol-year levels, adjusting for all variables in the model, are as follows: OR=2.96 (95% confidence interval [CI]=1.34-6.54) for 100 alcohol-years; OR=1.79 (95% CI=0.81-3.97) for 30 alcohol-years; OR=1.45 (95% CI=0.63-3.36) for 1 alcohol-year; and OR=1.44 (95% CI=0.62-3.34) for 0 alcohol-years.

Source: Calvert et al., 1992b. Used with permission from the author.

and cirrhosis (AST). Elevated levels of these enzymes may also be due to nonhepatic origins, such as myocardial infarction, acute pancreatitis (AST and ALT), or skeletal, cerebral, or renal necrosis (AST).

With respect to exposure to 2,3,7,8-TCDD, elevations in serum ALT and AST appear to be transient effects of acute exposure (Table 7-20). Case reports note that some populations have increased serum ALT levels shortly after exposure (Seveso children, British and Czechoslovakian TCP production workers) (May, 1973; Jirasek et al., 1974; Caramaschi et al., 1981; Mocarelli et al., 1986). Whereas epidemiologic studies conducted 10 to 30 years after last exposure reported no effects in exposed workers, Vietnam veterans and Missouri residents compared to unexposed control groups (Table 7-20) (Suskind and Hertzberg, 1984; May, 1982; Martin, 1984; Bond et al., 1983; Calvert et al., 1992; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Hoffman et al., 1986; Webb et al., 1989; Ott et al., 1993b), or workers with and without chloracne (Moses et al., 1984). Furthermore, it appears from a single study that current exposure to 2,3,7,8-TCDD-contaminated substances must exceed some threshold to produce enzyme elevations (Poland et al., 1971). Normal levels of AST (AST=10.6 U/L) were found in workers who volunteered to participate in a medical study conducted concurrently with their employment in a New Jersey chemical facility producing TCP and 2,4,5,-T (Poland et al., 1971). This was the same plant included in a later cross-sectional medical study of workers that found, in 1988, a high mean serum 2,3,7,8-TCDD level for the group (220 pg/g) but no elevations in AST or ALT (Calvert et al., 1992; Fingerhut et al., 1991a). Similarly, no increases in AST were noted in the BASF accident cohort (Ott et al., 1993b).

Several reports illustrate the probable transiency of ALT and AST elevations after heavy 2,3,7,8-TCDD exposure. Both enzymes were reported to be elevated among TCP production workers employed in Czechoslovakia who were described as exhibiting symptoms of "chemical intoxication" (Jirasek et al., 1974). The authors reported that AST and ALT levels were "different" in 11 (20%) of the 55 examined workers. These workers were exposed to 2,3,7,8-TCDD-contaminated chemicals, pentachlorophenol, and its production by-products, which include hexa-, hepta-, and octachlorinated dioxins and dibenzofurans. The exposures occurred between 1965 and 1968, with the ensuing effects beginning shortly

Table 7-20. Serum Alanine Aminotransferase (ALT) Among Seveso Children and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed N	Exposed Mean level[a]	Exposed (SD)	Unexposed N	Unexposed Mean level[a]	Unexposed (SD)
Mocarelli et al., 1986	Seveso children						
	1977 Boys	46[b]	12.25[c]	(7.14-20.99)	45[b]	9.33[c]	(3.73-23.33)
	Girls	100	12.97	(6.68-25.18)	141	11.99	(5.51-26.12)
	1982 Boys	33	10.74	(5.09-22.65)	39	10.74	(5.09-22.65)
	Girls	119	12.27	(6.5 -23.17)	136	12.19	(6.46-23.01)
Caramaschi et al., 1981	Seveso children	141[d]	3.5[e,c]		0[f]	0[e]	
Moses et al., 1984	TCP production workers; 10-30 years postexposure	105[d]	15.9	(13.0)	101[f]	15.7	(13.0)
Calvert et al., 1992	TCP production workers; 15-37 years postexposure	280[g]	33.8	(22.6)	259	33.0	(21.2)
Ott et al., 1993a	BASF accident cohort	133	14.8	(8.4)	6,721	15.1	(10.0)
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	134	---[h]		135	---[h]	
Webb et al., 1989	Missouri residents						
	<20[i]	16	22.7	(2.76)			
	20-60	12	22.5	(2.39)			
	>60	12	23.3	(3.06)			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army ground troops	2,490	26.4[j,k]		1,972	25.8	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[l]	339	19.16	---	777	20.34	
	Low 15-≤33.3[l]	191	20.83	---	---	---	
	High >33.3[l]	182	20.09[c]	---	---	---	

[a]ALT; units U/L.
[b]Number of samples.
[c]$p<0.05$.
[d]Chloracne.
[e]% Abnormal.
[f]No chloracne.
[g]% Abnormal: workers 4.3; unexposed 5.0; OR=0.85 (95% CI=0.38, 1.89).
[h]% Abnormal: exposed 6.0; unexposed 3.0.
[i]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[j]Geometric mean.
[k]% Abnormal; Vietnam veterans, 5.3; non-Vietnam veterans, 4.4; OR=1.2, 95% CI=0.9-1.5.
[l]Serum 2,3,7,8-TCDD in pg/g of lipid.

thereafter. The observation period lasted from 1967 to 1973. In a follow-up report of the same population conducted in approximately 1977, Pazderova-Vejlupkova et al. (1981) did not report liver enzyme levels but suggested that the levels were not abnormal.

Similarly, ALT was increased in 5 of 14 TCP workers from Great Britain who were in the manufacturing building at the time of a TCP reactor explosion in 1968 (May, 1973). Levels for AST were not reported. In 1977, workers from the same facility were reevaluated. No elevations in AST or ALT were found in production and laboratory workers with chloracne (May, 1982).

Finally, during the first year after the TCP reactor explosion, Caramaschi et al. (1981) evaluated AST and ALT among Seveso children with and without chloracne. Only ALT was statistically significantly elevated in children with chloracne. In a larger study, Mocarelli et al. (1986) tested liver enzyme levels yearly from 1977 to 1982 in male and female children from Seveso and from the unexposed surrounding area. In the 1977, 1979, 1980, and 1981 test series ALT, but not AST, was statistically significantly ($p<0.05$) elevated among male children in Seveso compared with unexposed comparisons. Female children had normal levels compared with controls for all years. In 1982, ALT levels in the exposed boys returned to normal.

None of the studies reporting elevations in ALT or AST identified clinical evidence of liver disease in the study populations. Therefore, in the absence of reports of hepatic or nonhepatic diseases related to changes in ALT or AST levels among exposed individuals, it is possible that the increases in ALT and AST are related to high-level, acute exposure to 2,3,7,8-TCDD-contaminated chemicals and that, barring additional exposure, the enzyme levels decrease with time.

7.13.2.6. *D-Glucaric Acid*

In five studies of Seveso residents, TCP production workers, and Vietnam veterans, urinary excretion of D-glucaric acid was measured to determine if exposure to 2,3,7,8-TCDD induced hepatic microsomal activity (Table 7-21). D-glucaric acid excretion is an indirect but valid indicator of enzyme induction.

Table 7-21. Mean D-Glucaric Acid Levels Among Seveso Residents, TCP Production Workers, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean	(SD)	N	Mean	(SD)
Ideo et al., 1985	Seveso adults Levels measured in 1978 (Zone B)	117	27.1[a,b,c]	---	127	19.8[a,b,d]	---
	Seveso children Zone A, 1976	14[e]	39[a,b,c]	---	17[f]	20.5[b]	---
	Zone B 1979 1980	 26.8 17.0	 ---[g] ---	 --- ---	 --- ---	 --- ---	 --- ---
May, 1982	TCP production workers in Great Britain	41	2.07[h]	---	31	1.52[h]	---
Martin, 1984	TCP production workers in Great Britain	39	2.09[c]	(0.7-7.9)	126	1.59	(0.8-8.3)
Calvert et al., 1992	TCP and 2,4,5-T production workers in Missouri and New Jersey	273	14.1[i]	(11.1)	256	13.2[i]	(7.9)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[j] Low 15-≤33.3 High >33.3	317 180 173	14.11[k,l] 14.43[l] 15.22[l]	--- --- ---	727 --- ---	14.11[k] --- ---	--- --- ---

[a]Units: μmol/g of creatinine.
[b]Median level.
[c]$p < 0.05$.
[d]Residents of unexposed community.
[e]Skin lesions.
[f]No skin lesions.
[g]d-Glucaric acid levels measured in 1979 were significantly higher than levels measured in 1980 ($p < 0.05$), no data presented.
[h]d-Glucaric acid/creatinine ratio.
[i]Units = μg/g of creatinine.
[j]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[k]Units = μM.
[l]Contrasted to unexposed comparison population.

Ideo and colleagues (1985) measured urinary D-glucaric acid in adults and children from all zones of Seveso and from nearby uncontaminated towns. Of adults tested in 1978, D-glucaric acid excretion was significantly elevated in adults residing in Seveso, Italy, at the time of the reactor explosion compared with residents of unexposed communities (Seveso = 27.1 μmol/g of creatinine vs. unexposed = 19.8 μmol/g of creatinine, $p<0.05$). No further studies of adults have been published. A series of studies evaluated D-glucaric acid excretion in Seveso children (Ideo et al., 1985) (Table 7-21). In 1976, the levels in children from Zone A with chloracne (39 μmol/g of creatinine) were significantly greater than in children without chloracne (20.5 μmol/g of creatinine). Additional studies, conducted until 1981, found significant yearly decreases in urinary D-glucaric acid excretion. By 1981, levels were within normal range.

D-glucaric acid-creatinine ratios were used to assess urinary excretion in TCP production workers tested within a year of suspension of TCP production and 10 years after a TCP reactor explosion. For exposed workers with or without chloracne at the time of the study, the D-glucaric acid-creatinine ratio was significantly higher than that of the unexposed controls (exposed = 2.09; unexposed controls = 1.59; $p<0.05$) (Martin, 1984).

Other studies of Air Force Ranch Hands or TCP production workers that examined D-glucaric acid excretion did not find increases in exposed populations 10 to 37 years after last exposure to 2,3,7,8-TCDD-contaminated chemicals (Roegner et al., 1991; Calvert et al., 1992).

7.13.2.7. *Comment*

The evidence presented by the large number of case reports and epidemiologic studies of groups exposed to 2,3,7,8-TCDD-contaminated chemicals suggests that hepatic enzyme induction occurred in some populations within a short time after high-level 2,3,7,8-TCDD exposure. In most cases, enzyme levels decreased as the time from exposure increased. However, even after more than 15 years since last exposure, levels of GGT continue to be significantly elevated in relation to serum 2,3,7,8-TCDD in TCP production workers with above-average alcohol consumption (Calvert et al., 1992) and in Air Force Ranch Hands (Roegner et al., 1991). Other Vietnam veterans, U.S. Army ground troops, also have

significantly increased GGT levels compared with non-Vietnam veterans, but this increase in the Army veterans is probably not due to exposure to high levels of 2,3,7,8-TCDD. In the population of Army Vietnam veterans studied, the mean serum 2,3,7,8-TCDD was approximately 4 pg/g (Centers for Disease Control Veterans Health Studies, 1988), compared to a mean of 220 pg/g (range to 3,400 pg/g) in the production workers and a median of 12 pg/g (range to 600 pg/g) in the Ranch Hands.

The finding of continued elevation of GGT may be a spurious result or it may reflect activity related to the continued presence of above-background levels of 2,3,7,8-TCDD in exposed individuals.

7.13.2.8. *Porphyrin Metabolism*

In rats and mice, exposure to 2,3,7,8-TCDD has been clearly shown to produce alterations in porphyrin metabolism (Goldstein et al., 1973, 1982; Smith et al., 1982; Jones et al., 1981; DeVerneuil et al., 1983; Cantoni et al., 1981). Whether 2,3,7,8-TCDD is associated with porphyrin changes in humans, particularly porphyria cutanea tarda (PCT), is a subject of some debate. PCT is a form of acquired or inherited porphyria caused by a deficiency of the enzyme uroporphyrinogen decarboxylase and the resulting overproduction and excretion of uroporphyrin (Sweeney, 1986). The predominant characteristics of PCT include skin fragility, blistering upon sun exposure, dark pigmentation, excess hair growth, hepatomegaly, reddish-colored urine, and urinary excretion of uro- and heptacarboxyl-icoporphyrins (Strik, 1979). PCT has been associated with excessive alcohol intake, oral estrogens, iron overload, hepatomas, and exposure to polyhalogenated hydrocarbons (Strik, 1979). A particularly large outbreak of PCT occurred after consumption of grain treated with hexachlorobenzene (HCB) (Cam and Nigogosyan, 1963). Cases of PCT were described in two populations of TCP production workers (Bleiberg et al., 1964; Jirasek et al., 1974) and among members of a family with inherited uroporphyrin decarboxylase deficiency who were living in Seveso at the time of the reactor explosion (Strik, 1979).

In 1964, Bleiberg reported that based on the Watson-Schwartz test, 11 of 29 New Jersey TCP production workers with chloracne had porphyria cutanea tarda as a result of increased urinary uroporphyrins, coproporphyrins, and urobilinogen. In a later study of 73

workers from the same plant in New Jersey, including four of the individuals that Bleiberg et al. (1964) found to have elevated urinary porphyrins, Poland et al. (1971) identified one individual with uroporphyrinuria. The report did not explain if this individual was one of the four described by Bleiberg et al. (1964). In the NIOSH study that examined workers from the same plant in New Jersey, the pattern of urinary porphyrin excretion for each participant was assessed to determine the presence of PCT (Calvert et al., 1993). No difference was found between workers and an unexposed control group in the prevalence of PCT (OR=0.93, 95% CI=0.19, 4.54). Furthermore, there were no differences in the risk between workers and the control group for an out-of-range uroporphyrin concentration or an out-of-range coproporphyrin concentration. Because this study was conducted at least 15 years after last occupational exposure to TCDD, it was not possible to determine whether porphyrinuria occurred during the years more proximal to occupational 2,3,7,8-TCDD exposure.

There is some question as to the appropriateness of the clinical test Bleiberg et al. (1964) used to measure porphyrin levels. The Watson-Schwartz test was capable of measuring only the presence of porphobilinogen. The test was rarely positive in cases of exposure to hepatotoxins. Bleiberg's findings suggest that either other unspecified tests were used to measure uro- and coproporphyrin levels or the authors misinterpreted the function of the Watson-Schwartz test.

Jirasek et al. (1974) found 11 of 55 Czechoslovakian TCP production workers to have elevated urinary uroporphyrins that decreased during the observation period; the authors did not describe the test used to measure urinary uroporphyrins or coproporphyrins. Ten years later in a follow-up study, Pazderova-Vejlupkova et al. (1981) found no evidence of increased excretion of uroporphyrins or dermatological indications of PCT in the same group of workers.

There is some question that the porphyria noted in the New Jersey (Bleiberg et al., 1964) and Czechoslovakian workers was due to 2,3,7,8-TCDD exposure. Jones and Chelsky suggest that the observed cases of PCT in both plants may be due to exposure to HCB or a combination of both 2,3,7,8-TCDD and HCB (Jones and Chelsky, 1986). HCB was manufactured at the New Jersey plant from 1951 until 1960 and was produced at the facility

in Czechoslovakia during the production of pentachlorophenol and TCP (Jirasek et al., 1973). Evidence of porphyria in other studies of individuals exposed to 2,3,7,8-TCDD-contaminated substances is minimal. Although Suskind and Hertzberg (1984) sampled urine for porphyrins in the examination of West Virginia TCP workers, the authors report that the data are not valid. However, there was no dermatologic evidence of porphyria identified among the exposed workers. Moses et al. (1984) found no difference in porphyrin levels when comparing TCP workers with and without chloracne. Finally, no PCT was reported among three laboratory workers exposed to pure 2,3,7,8-TCDD (Oliver, 1975), the only humans known to have documented unintentional exposure to uncontaminated 2,3,7,8-TCDD.

In 1977, 60 Seveso residents were tested for elevated porphyrins, exclusive of the family with inherited deficiency of uroporphyrinogen decarboxylase. None of the 60 residents developed PCT; however, 13 (22%) exhibited secondary coproporphyrinuria, 5 of whom showed a slight increase of urocarboxyporphyrins, heptacarboxyporphyrins, and coproporphyrins classified as a "transition constellation to CHP type A" (Doss et al., 1984). Porphyrin levels were retested in 1980. Porphyrin levels returned to normal in 12 individuals. In three of those with transition CHP, porphyrin levels were higher than those in 1977 and were attributed to liver damage and alcohol consumption. Doss et al. (1984) suggested that in the Seveso family with uroporphyrinogen dicarboxylase deficiency, the exposure to 2,3,7,8-TCDD-contaminated effluent caused an exacerbation of a preexisting enzyme deficiency.

7.13.2.9. *Comment*

It is possible that the PCT and elevated urinary porphyrins observed in the New Jersey and Czechoslovakian workers were a direct result of exposure to hexachlorobenzene. In the follow-up studies, urinary porphyrin levels in workers were not elevated (Pazderova-Vejlupkova et al., 1981; Poland et al., 1971) or did not differ from levels in the control group (Calvert et al., 1992). The transient elevations in coproporphyrins among 22 Seveso residents described by Doss et al. (1984) may be a direct result of acute exposure to 2,3,7,8-TCDD.

The association is not clear. However, 2,3,7,8-TCDD is a potent porphyrigen in rats and mice and, therefore, high acute exposures may have contributed to the observed changes in porphyrin levels in these populations.

7.13.2.10. *Lipid Levels*

Animal studies provide conflicting evidence on the relationship between exposure to 2,3,7,8-TCDD and serum lipid levels. Some studies suggest that short-term high exposure to 2,3,7,8-TCDD increases serum cholesterol (Greig et al., 1973; Zinkl et al., 1973; Poli et al., 1980; Gasiewicz et al., 1980; Schiller et al., 1986; Gasiewicz and Neal, 1979; Olson et al., 1980) and triglyceride fractions (Schiller et al., 1986; McConnell et al., 1978a, b; Gasiewicz and Neal, 1979), whereas other studies suggest a decrease (Gasiewicz et al., 1980; Olson et al., 1980) or no change in triglyceride levels (Poli et al., 1980). The human data appear to be similarly confusing. A number of case reports and epidemiologic studies have described increases in the levels of serum lipid fractions, particularly total cholesterol and triglycerides, in TCP production workers, laboratory workers, Seveso and Missouri residents, and Vietnam veterans. Others report no differences between subject and reference levels. A summary of the reported levels is included in Tables 7-22 and 7-23.

7.13.2.11. *Total Cholesterol*

Two case reports of workers with presumably high exposures to 2,3,7,8-TCDD-contaminated chemicals described elevations in total cholesterol. In 50% of 55 Czechoslovakian TCP production workers examined between 1968 and 1969 who exhibited signs of "chemical intoxication," total cholesterol was noted as elevated (Jirasek et al., 1974). In a follow-up study 10 years later, lipid levels among workers removed from exposure were not significantly different from referent levels, but total cholesterol levels remained significantly increased (Pazderova-Vejlupkova et al., 1981). In a separate report, three laboratory workers who were exposed during the synthesis of pure 2,3,7,8-TCDD developed serum cholesterol levels in excess of 7.7 mmol/L (Oliver, 1975). No information on pre-exposure cholesterol levels was provided in either report.

Table 7-22. Mean Total Cholesterol Levels Among Seveso and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean[a] level	(SD)	N	Mean[a] level	(SD)
Mocarelli et al., 1986	Seveso children						
	1977	16[b]	4.62	3.26-5.98	28[b]	4.45	(3.12-5.77)
	1982	182[b]	4.48	2.47-5.99	250[b]	4.41	(2.99-5.83)
Caramaschi et al., 1981	Seveso children	138	15.2[c,d]	---	120	12.5[c,e]	---
Assennato et al., 1989	Seveso residents						
	1976	193[d]	4.78	(0.99)	---[f]	---	---
	1982	152[d]	4.06	(0.80)	123[e]	4.14	(0.77)
	1984	142[d]	4.09	(0.88)	196[e]	4.12	(0.86)
	1985	141[d]	4.14	(0.91)	167[e]	4.13	(0.78)
May, 1982	TCP production workers in Great Britain	41	5.97	---	31	6.6	---
Martin, 1984	TCP production workers in Great Britain	39	6.02[g]	---	126	5.6	---
Poland et al., 1971	TCP production workers in New Jersey	71	6.12	(0.82)	---	---	---
Moses et al., 1984	TCP production workers in West Virginia	105[d]	5.38	(0.88)	101[e]	5.37	(0.85)

[a]Units = mmol/L.
[b]Number of samples.
[c]% abnormal.
[d]Chloracne.
[e]No chloracne.
[f]No data for controls in 1976.
[g]$p < 0.05$.

Table 7-22. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean[a] level	(SD)	N	Mean[a] level	(SD)
Suskind and Hertzberg, 1984	TCP production workers in West Virginia	200	5.46	(0.07)	163	5.28	(0.08)
	TCP production workers: Chloracne vs. never chloracne	105[d]	5.44	(0.08)	28[e]	5.30	(0.18)
Ott et al., 1993b	BASF accident cohort	135	6.14[m]	(1.01)	6,581	6.37[m]	(1.17)
Calvert et al., 1993	TCP and 2,4,5-T production workers in Missouri and New Jersey	278	5.7[n]	---	259	5.6[n]	---
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	142	4.97[g]	(0.96)	148	5.2	(1.09)
Webb et al., 1989	Missouri residents						
	<20[h]	16	5.88	(1.10)	---	---	---
	20-60[h]	12	6.60	(0.93)	---	---	---
	>60[h]	12	6.76	(0.97)	---	---	---
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	5.43[i,j]	---	1,972	5.36	---
Roegner et al., 1991	Air Force Ranch Hand personnel						
	Unknown ≤10[k]	338[l]	5.53	---	777	5.51	---
	Low 15-≤33.3[k]	191	5.55	---	---	---	---
	High >33.3[k]	182	5.68[g]	---	---	---	---

[h]Adipose tissue levels of 2,3,7,8-TCDD in pg/g of lipid.
[i]Geometric mean.
[j]% abnormal: Vietnam veterans, 5.1; non-Vietnam veterans, 4.7; OR=1.1, 95% CI=0.8-1.5.
[k]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[l]Contrasted to unexposed comparisons.
[m]Adjusted for age, body mass index, smoking history.
[n]Adjusted for age, body mass index, smoking, gender.

Table 7-23. Mean Triglyceride Levels Among Seveso and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean[a]	(SD)	N	Mean[a]	(SD)
Mocarelli et al., 1986	Seveso children 1977 1982	 38[b] 207[b]	 0.97 0.91	 (0.6-1.50) (0.52-1.60)	 36[b] 257[b]	 0.95 0.86	 (0.63-1.51) (0.47-1.56)
Assennato et al., 1989	Seveso residents 1976 1982 1984 1985	 193 152 142 141	 0.99 0.87 0.94 0.84	 (0.43) (0.40) (0.59) (0.44)	 --- 123 196 167	 --- 0.85 0.88 0.87	 --- (0.37) (0.46) (0.55)
May, 1982	TCP production workers in Great Britain	41[d]	2.03	---	31[e]	1.83	---
Martin, 1984	TCP production workers in Great Britain	39[d]	1.97[f]	(0.4-4.0)	126[e]	1.41	(0.3-3.2)
Moses et al., 1984	TCP production workers in West Virginia	93[d]	1.69[g]	(1.26)	93[e]	1.46	(0.73)
Suskind and Hertzberg, 1984	TCP production workers in West Virginia	200	1.65	(0.08)	163	1.76	(0.08)
Ott et al., 1993b	BASF accident cohort	135	1.91[h]	(1.19)	4,471	1.97[h]	(1.65)
Calvert et al., 1993	TCP and 2,4,5-T production workers in Missouri and New Jersey	273	1.20	(---)	259	1.15[i]	(---)

[a]Units = mmol/L.
[b]Number of samples.
[c]Adipose tissue 2,3,7,8-TCDD levels in pg/g of lipid.
[d]Chloracne.
[e]No chloracne.
[f]$p < 0.01$.
[g]$p = 0.056$.
[h]Adjusted for age, body mass index, smoking history.
[i]Adjusted for age, body mass index, smoking, gender.

Table 7-23. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean[a]	(SD)	N	Mean[a]	(SD)
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	141	1.07	(0.73)	146	1.19	(1.07)
Webb et al., 1989	Missouri residents						
	<20[c]	16	2.17	(2.08)	---	---	---
	20-60[c]	12	1.81	(1.19)	---	---	---
	>60[c]	12	2.69	(1.06)	---	---	---
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.06[j,k]	---	1972	1.05	---
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[l]	338	1.02[f,m]	---	777	1.16	---
	Low 15-≤33.3[l]	191	1.37[f,m]	---			
	High >33.3[l]	182	1.35[f,m]	---			

[j]% Abnormal: Vietnam veterans, 4.7; non-Vietnam veterans, 5.3; OR=0.9, 95% CI=0.7-1.2.
[k]Geometric mean.
[l]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[m]Contrasted to unexposed comparison population.

The results of epidemiologic studies conflict. Among British TCP production workers whose last exposure to 2,3,7,8-TCDD-contaminated chemicals was less than 1 year at the time of the study, total cholesterol levels in exposed workers with (6.02 mmol/L) and without (6.14 mmol/L) chloracne were significantly elevated compared to unexposed controls (5.6 mmol/L) (Martin, 1984) (Table 7-22), whereas May (1982) found unexposed workers (6.6 mmol/L) to have cholesterol levels higher than those of exposed workers with chloracne (5.97 mmol/L). Martin (1984) also found reduced, but not significantly, HDL cholesterol among exposed workers with chloracne (1.19 mmol/L) compared to unexposed controls (1.25 mmol/L). The differences in the results may be due to differences in the control groups and inclusion of different workers in the exposed groups.

Cholesterol levels in West Virginia TCP production workers were compared with unexposed workers from the same plant (Suskind and Hertzberg, 1984). No difference was identified in mean cholesterol levels between workers and controls. However, when lipid fractions were examined, there was a larger but nonsignificant percentage of exposed workers with elevated LDL cholesterol (7.7%) compared to unexposed controls (6.3%). A comparison of workers with persistent chloracne, no chloracne, or a history of chloracne found a significant association ($p < 0.05$) between the proportion of out-of-range LDL cholesterol values and persistent chloracne. An out-of-range LDL was defined as above the 90th percentile of the total range of values. In a second study that compared West Virginia workers with and without chloracne, no difference was found in mean cholesterol levels (Moses et al., 1984). In the NIOSH study, there was little difference between the adjusted mean total cholesterol levels for workers (5.7 mmol/L) and referents (5.6 mmol/L) and no relation to increasing serum 2,3,7,8-TCDD levels (Calvert et al., 1993). The mean levels were adjusted for age, body mass index, age, and gender.

Mean cholesterol levels were no different between workers in the BASF accident cohort (6.14 mmol/L) and the referent population (6.37 mmol/L) and were not related to current or log TCDD back-calculated levels (Ott et al., 1993b). In addition, no significant differences were noted between the exposed and unexposed populations for HDL and LDL levels.

In general, cholesterol levels among exposed community residents were not increased. Despite their high exposure to 2,3,7,8-TCDD-contaminated TCP, neither children nor adults from Seveso were found to have elevated serum cholesterol levels compared to controls (Mocarelli et al., 1986; Assennato et al., 1989). Evaluated from 1976 through 1985, cholesterol levels in this population remained constant throughout the study period (Table 7-22). Similarly, among Missouri residents, serum cholesterol was not related to residence in the Quail Run Mobile Home Park (Hoffman et al., 1986) or to adipose tissue 2,3,7,8-TCDD levels (Webb et al., 1989).

Among U.S. Army veterans, there was no difference in total cholesterol levels between groups serving in Vietnam or other arenas (Centers for Disease Control Vietnam Experience Study, 1988a). In contrast, there was a statistically significant positive relationship between Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g and total cholesterol in Air Force Ranch Hands (Roegner et al., 1991). The total cholesterol-HDL ratio was also highest in this serum 2,3,7,8-TCDD category.

7.13.2.12. *Triglycerides*

Elevated triglyceride levels were reported only in three of the studied populations. Among British TCP workers, triglycerides were significantly higher in exposed workers with (1.97 mmol/L) and without (1.90 mmol/L) chloracne compared to unexposed controls (1.41 mmol/L) (Martin, 1984). TCP production workers from West Virginia who had chloracne had statistically nonsignificant increase in mean triglyceride levels (chloracne = 1.69 mmol/L; without chloracne = 1.46 mmol/L) (Moses et al., 1984). In addition, compared to the unexposed background population, triglyceride levels in Air Force Ranch Hands were significantly elevated for all serum 2,3,7,8-TCDD categories (Table 7-23). Among workers in the NIOSH study there appeared to be a small rise in triglyceride levels with increasing serum 2,3,7,8-TCDD (Calvert et al., 1993). The mean adjusted triglyceride levels and the percent of abnormal triglyceride values increase with increasing serum 2,3,7,8-TCDD level ($<$158 femtograms/liter [fg/L], mean = 1.04 mmol/L, % abnormal = 5.7; 158-520 fg/L, mean = 1.26 mmol/L, % abnormal = 6.1; 521-1,515 fg/L, mean = 1.23 mmol/L, % abnormal = 6.1; 1,516-19,717 fg/L, 1.35 mmol/L, % abnormal = 1.7, $p<0.05$ compared

to referents [1.15 mmol/L]). Odds ratios and 95% confidence intervals for the quartiles are OR=0.7 (95% CI=0.5, 12.0), OR=1.1 (95% CI=0.4, 3.2), OR=0.9 (95% CI=0.3, 2.9), and OR=1.7 (95% CI=0.6, 4.6), respectively. The authors suggest that despite this small rise with 2,3,7,8-TCDD level, the influence of factors such as gender, body mass index, use of beta-blocker medication, and smoking had far greater effects on lipid concentration than did 2,3,7,8-TCDD level. Similarly, triglyceride levels in the BASF accident cohort were similar to those in the referent cohort and not related to 2,3,7,8-TCDD level (Ott et al., 1993b).

Triglyceride levels were not elevated in Missouri (Hoffman et al., 1986; Webb et al., 1989) or Seveso residents (Mocarelli et al., 1986; Assennato et al., 1989) or in U.S. Army Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a).

7.13.2.13. *Comment*

The effect of exposure to 2,3,7,8-TCDD-contaminated chemicals on cholesterol or triglyceride levels is not consistently well defined in the available studies. It is possible the transient elevations in total cholesterol and triglyceride levels may have occurred after high 2,3,7,8-TCDD exposure, as in the experience of the British and Czechoslovakian TCP workers and British laboratory workers. However, this scenario does not concur with the evidence from Seveso or among Ranch Hands. Despite their very high exposure to 2,3,7,8-TCDD-contaminated chemicals, neither adults nor children from Seveso had lipid levels above the referent level. In contrast, Ranch Hands continue to have elevated lipid levels despite the extended length of time between exposure and testing. Other factors, such as dietary fat intake, familial hypercholesterolemia, alcohol consumption, and exercise, which also affect cholesterol and other lipid levels, may be factors that were not considered in many of these studies.

In the 1992 phase of the Ranch Hand study, additional parameters that may affect lipid levels were examined. It is hoped that this new information will help clarify the increased levels observed in the Ranch Hand population.

7.13.3. Other Gastrointestinal Disorders

A variety of gastrointestinal disorders other than liver conditions were reported among TCDD-exposed groups. After heavy, acute, or chronic exposure, chemical workers in West Virginia (Ashe and Suskind, 1950), West Germany (Baader and Bauer, 1951; Bauer et al., 1961), and Czechoslovakia (Jirasek et al., 1974) consistently reported transient episodes of right upper quadrant pain, loss of appetite, and nausea. None of the reports suggest an etiology for these symptoms nor were the symptoms reported in later follow-up studies of any cohorts (Suskind and Hertzberg, 1984; Moses et al., 1984; Pazderova-Vejlupkova et al., 1981).

Three investigations of TCP production workers reported an increased prevalence of a history of upper gastrointestinal tract ulcer across all age strata of West Virginia workers (exposed = 20.7% vs. unexposed = 5.5%) (Suskind and Hertzberg, 1984) and all digestive system diseases (type not specified) among workers employed in a plant in Midland, Michigan (prevalence: exposed = 1.5% vs. unexposed = 0.5%) (Bond et al., 1983). The factors contributing to these conditions have not been examined fully. Neither the Ranch Hand study (Roegner et al., 1991) nor the NIOSH study (Calvert et al., 1992) found increased risk of upper gastrointestinal tract ulcers with increasing serum TCDD level.

7.13.4. Thyroid Function

The thyroid plays an essential role in the maintenance of metabolic rate, food intake, and differentiation and maturation of various cell types. Because many of the toxic effects of 2,3,7,8-TCDD noted in animals resemble the signs of thyroid dysfunction, researchers considered the role of the thyroid in 2,3,7,8-TCDD toxicity (Neal et al., 1979). Some studies found a single high dose of 2,3,7,8-TCDD resulted in decreased levels of serum thyroxine (T4), indicating hypothyroidism, but no consistent findings were reported for alterations in 3,5,3′-triiodothyronine (T3); researchers report decreases, no change, and increases in levels of T3 (Bastomsky, 1977; Potter et al., 1983; Pazdernik and Kozman, 1985; Potter et al., 1986; Henry and Gasiewicz, 1987; Roth et al., 1988; Muzi et al., 1989). Furthermore, hypothyroidism induced in rats was protective against 2,3,7,8-TCDD-induced weight loss, immunotoxicity, and mortality (Rozman et al., 1984; Pazdernik and Kozman,

1985). This protective effect was reversed when T4 supplements were given to these animals (Rozman et al., 1985). A recent study by Henry and Gasiewicz (1987), however, found that in hamsters serum T3 and T4 levels increased after 2,3,7,8-TCDD administration, putting in question the role of the thyroid in 2,3,7,8-TCDD-induced toxicity.

These animal findings suggest that if the thyroid plays a role in human toxicity, hypothyroidism would be manifested as a reduction in serum T4; in extreme cases of 2,3,7,8-TCDD toxicity, however, one may experience hyperthyroidism. Only two studies of production workers examined this issue (Suskind and Hertzberg, 1984; Ott et al., 1993b). Suskind and Hertzberg (1984) performed T4 radioimmunoassay and thyroxine-binding globulin (TBG) tests and found no significant differences between exposed and unexposed workers. Quantitative results were not presented. Similarly, TSH, T4, and TBG levels were within normal range in the BASF accident workers, although TGB levels were positively related to 2,3,7,8-TCDD levels (Ott et al., 1993b). The Ranch Hand study indicated a reduction of T3% uptake (Table 7-24) and an increase in the mean level of thyroid-stimulating hormone (TSH) (Table 7-25) with increasing serum 2,3,7,8-TCDD level; these results, however, did not reach statistical significance. Among Army Vietnam veterans, mean TSH levels, but not mean free thyroxine index (FTI) levels, were statistically significantly higher than among non-Vietnam veterans, after adjustment for the six entry characteristics of age and year of enlistment, race, enlistment status, general technical test score, and primary military occupation (Table 7-24) (Centers for Disease Control Vietnam Experience Study, 1988a). However, the percent of values that were out-of-reference range did not differ significantly for TSH (Vietnam veterans, 1.0%; non-Vietnam veterans, 0.6%, OR=2.0, 95% CI=0.9-4.3) and FTI (Vietnam veterans, 5.4%; non-Vietnam veterans, 4.6%, OR=1.2, 95% CI=0.9-1.5). The exposure levels were low, based on the sample for which 2,3,7,8-TCDD was measured.

The most recent study was conducted among infants in The Netherlands (Pluim et al., 1992). In a letter to the editor, Pluim and colleagues (1992) examined thyroid function among term breast-fed infants in relation to the total toxic equivalents per kg of breast milk fat (TEQ/kg) of dioxins (congeners were not specified). The authors measured T4, TBG, and TSH levels sequentially in infants at birth, at 1 week of age, and at 11 weeks of age (Tables

Table 7-24. Levels of Triiodothyronine Percent (T3%) Uptake or Free Thyroxine Index in Vietnam Veterans

Outcome			Exposed			Unexposed	
T3% uptake	Author	Population	N	Mean level	Adjusted RR	N	Mean level
	Roegner et al., 1991	U.S. Air Force Ranch Hand personnel					
		Unknown ≤10[a]	338	30.67	1.14	772	30.66
		Low 15-≤33.3[a]	194	30.36	0.93		
		High >33.3[a]	181[b]	29.99	0.45		
Free thyroxine index							
	Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	2.2[c]	Adjusted OR (95% CI) 1.2 (0.9,1.5)	1,972	2.2[c]

[a]Serum 2,3,7,8-TCDD in pg/g of lipid.
[b]$p < 0.05$ comparison of veterans at background level with ≥33.3 pg/g TCDD.
[c]Geometric mean.

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Table 7-25. Levels of Thyroid-Stimulating Hormone (TSH) in Vietnam Veterans, Nursing Infants, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level[a]		N	Mean level[a]	SD
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel			Adjusted RR (95% CI)			
	Unknown ≤10[b]	338	0.948	1.45 (0.62,3.40)	616	0.920	---
	Low 15-≤33.3[b]	194	0.978	0.88 (0.24,3.02)			
	High >33.3[b]	181	1.026[c]	2.15 (0.80,5.79)			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans			Adjusted OR (95% CI)			
		2,490	1.6[d]	2.0 (0.9-4.3)	1,972	1.6[d]	---
Pluim et al., 1992	Neonates; The Netherlands						
	At birth	11[e]	11.9	1.9[f]	14[g]	10.4	1.3[f]
	1 wk postnatal	11	2.56	0.41	15	2.93	0.41
	11 wks postnatal	12	2.50[c]	0.26	18	1.81	0.19
Ott et al., 1993b	BASF chemical workers	130	1.19 (0.90)[h]	---	---[i]	---	---

[a]Units: μU/ml.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]$p < 0.05$ compared to low-exposure group.
[d]Geometric mean.
[e]High-exposure group: 29.2-62.7 ng toxic equivalents/kg (toxic equivalencies [TEQ] per kg milk fat).
[f]Standard error of the mean.
[g]Low-exposure group: 8.7-28 ng TEQ/kg.
[h]Standard deviation.
[i]No referent values.

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7-25 and 7-26). Infants were classified in the high-exposure group if the breast milk contained 29.2-62.7 ng TEQ/kg and in the low-exposed group if the breast milk contained less than 28.0 ng TEQ/kg. At 1 week and 11 weeks postnatal, T4 and T4/TBG ratios were significantly higher among infants in the "high-exposure group." At 11 weeks, TSH was also higher in infants ingesting milk with the higher concentration of dioxins. The authors suggest that exposure to high levels of dioxins either in utero or through breast milk modulates the hypothalamic-pituitary-thyroid regulatory system of the infant (Pluim et al., 1992).

In summary, experimental animal studies provide some evidence that thyroid may mediate, at least in part, 2,3,7,8-TCDD toxicity. These results were based on animals receiving a single high dose of 2,3,7,8-TCDD. In addition, studies were performed in only two species of animals, and the effects may be species specific. The applicability of these findings to humans, therefore, needs further evaluation.

7.13.4.1. *Comment*

Few human studies examined the relationship between 2,3,7,8-TCDD exposure and thyroid function, and the results of present research are equivocal. These studies examined individuals with relatively lower exposure to 2,3,7,8-TCDD than the animals, and exposure was chronic. Little or no information has been reported for the effects specifically in production workers or Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels (Fingerhut et al., 1991a; Mocarelli et al., 1991). The data from the study of Ranch Hands, which measured serum 2,3,7,8-TCDD levels, suggest that in adults there are few long-term effects. Although the study of nursing infants suggests that ingestion of breast milk with a higher total TEQ may alter thyroid function, the study is limited by small numbers and a short observation period (Pluim et al., 1992).

The variety of ways in which 2,3,7,8-TCDD may affect thyroid function in humans makes it difficult to predict the net outcomes in terms of individual laboratory tests. It is possible that different relationships may exist among thyroid function indicators, depending on the exposure level of the individual.

Table 7-26. Levels of Thyroxine-Binding Globulin (TBG), Thyroxine (T4), or T4/TBG in Nursing Infants and BASF Accident Cohort

Outcome			Exposed[a]			Unexposed[b]		
TBG (nmol/L)	Author	Population	N	Mean level	Standard deviation	N	Mean level	Standard deviation
	Pluim et al., 1992	Neonates; The Netherlands						
		At birth	15	589.5	30.5[d]	18	520.1	27.2[d]
		1 wk postnatal	19	546.2	19.1	19	532.6	16.3
		11 wks postnatal	16	500.7	13.0	18	519.0	29.4
T4 (nmol/L)								
		Neonates; The Netherlands						
		At birth	15	134.3	4.8[d]	18	122.5	4.1[d]
		1 wk postnatal	19	178.7[c]	5.5	19	154.5	6.3
		11 wks postnatal	16	122.2[c]	3.0	18	111.1	4.0

[a]High-exposure group: 29.2-62.7 ng toxic equivalents/kg (TEQ per kg milk fat).
[b]Low-exposure group: 8.7-28 ng TEQ/kg.
[c]$p<0.05$.
[d]Standard error of the mean.

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Table 7-26. (continued)

Outcome	Author	Population	Exposed			Unexposed		
TBG (nmol/L)			N	Mean level	Standard deviation	N	Mean level	Standard deviation
T4/TBG								
	Pluim et al., 1992	Neonates; The Netherlands						
		At birth		0.232	0.008[d]	18	0.240	0.007[d]
		1 wk postnatal		0.332[c]	0.011	19	0.291	0.009
		11 wks postnatal		0.247[c]	0.009	18	0.220	0.008
TBG (mg/L)	Ott et al., 1993b	BASF accident cohort	145	12.7	3.2	42[e]	12.7	2.9
T4 (μg/dL)	Ott et al., 1993b	BASF accident cohort	144	7.8	1.9	42[e]	8.3	1.5
T4/TBG (mU/L)	Ott et al., 1993b	BASF accident cohort	131	6.3	1.3	42[e]	6.6	1.6

[e]Source: Zober et al., 1992.

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7.13.5. Diabetes

Diabetes and fasting serum glucose levels were evaluated in cross-sectional medical studies due to the apparently high prevalence of diabetes and abnormal glucose tolerance tests in one case report of 55 TCP workers (Pazderova-Vejlupkova et al., 1981). In this group, evaluated 10 years after exposure ended, approximately 50% of the subjects had either confirmed cases of diabetes or abnormal glucose tolerance tests.

The results of later medical studies are mixed. Cross-sectional studies of workers from Nitro, West Virginia (Suskind and Hertzberg, 1984; Moses et al., 1984), found no difference in glucose levels between the exposed and control populations, although no quantitative values were presented in either study. Similarly, the adjusted odds ratio for out-of-range fasting glucose levels comparing Vietnam veterans to non-Vietnam veterans was not statistically significant (OR=1.0, 95% CI=0.4-2.2). But a comparison of the adjusted mean fasting glucose levels between the two groups was marginally significant (Vietnam veterans, 5.2 mmol/L; non-Vietnam veterans, 5.1 mmol/L, $p<0.05$) (Centers for Disease Control Vietnam Experience Study, 1988a). Mean fasting glucose levels in the BASF accident cohort were marginally elevated compared to the referent population and associated with current levels of 2,3,7,8-TCDD ($p=0.062$) but not the back-extrapolated level (Ott et al., 1993b). Results from the NIOSH (Sweeney et al., 1992) and Ranch Hand studies (Roegner et al., 1991) more strongly suggest that serum 2,3,7,8-TCDD levels may be positively and significantly related to diabetes and fasting serum glucose levels.

In the NIOSH study, the relationship between exposure to 2,3,7,8-TCDD and possible alterations in glucose metabolism was assessed using two outcome measures: case definition of diabetes and fasting serum glucose levels. Participants met the case definition of diabetes if their fasting serum glucose level was 7.8 mmol/L or greater on two consecutive occasions (National Diabetes Data Group, 1979) or if they reported a positive history of physician-diagnosed diabetes after the date of first employment at the plant. Based on logistic regression, the risk of diabetes was found to increase by approximately 12% for every 100 pg/g 2,3,7,8-TCDD ($p<0.001$) (Table 7-27). Adjusted fasting serum glucose levels in workers and referents were also compared in a separate analysis using linear regression. Fasting serum glucose was 10% higher in workers with 1,000 pg/g 2,3,7,8-TCDD than in

Table 7-27. Multiple Logistic Regression Model for Cases of Diabetes[a] for TCP Production Workers

Variable	Beta	Standard error	Odds ratio[b]	p-Value[c]
Intercept[d]	-8.8913	1.7131	--	0.001
Serum TCDD	0.00114	0.0003	1.12	<0.001
Age	0.0776	0.0204	2.17	<0.001
Body mass index	0.0996	0.0370	1.65	0.007
Race 0=other, 1=white	-1.6659	0.4706	0.19	<0.001
Family history of diabetes (0=no, 1=yes)	0.6957	0.3641	2.01	0.056

[a]History of physician-diagnosed diabetes (diagnosed after exposure to 2,3,7,8-TCDD-contaminated chemicals) or fasting serum glucose level of 7.8 mmol/L on 2 consecutive days.
[b]Odds ratios for continuous variables are based on the difference of 100 pg per gram of lipid of TCDD, 10 years of age, and 5 kg/m^2 body mass index.
[c]p-Values are based on Wald Chi-square statistic.
[d]Number of observations = 530.

Source: Sweeney et al., 1992.

workers and referents with 20 pg/g 2,3,7,8-TCDD ($p<0.001$) (Table 7-28). Yet, based on the magnitude of the regression coefficients, age, and body mass index (kg/m^2), both known risk factors for diabetes appear to have greater influence on the increases in both the risk of diabetes and elevated fasting serum glucose levels than 2,3,7,8-TCDD level.

In the Ranch Hand study, diabetic status was assessed by measuring fasting serum glucose and 2-hour postprandial glucose and by using a case definition of diabetes. Diabetes was defined as having a verified history of diabetes or an oral glucose tolerance test of ≥ 11.1 mmol/L (200 mg/dL) (Roegner et al., 1991). The analyses of all three parameters suggest a consistent association between serum 2,3,7,8-TCDD levels above 33.3 pg/g and an increased risk of diabetes. Adjusted relative risks comparing Ranch Hands with serum 2,3,7,8-TCDD above 33.3 pg/g and the unexposed comparison group were statistically significantly elevated for fasting serum glucose levels and diabetes (glucose, RR=2.95, $p<0.001$; diabetes, RR=2.51, $p<0.001$) (Table 7-29) and for the 2-hour postprandial glucose test (RR=2.35, $p<0.035$). In addition, Ranch Hand personnel meeting the case definition for diabetes were also more likely to have earlier onset of diabetes than the unexposed comparisons (Wolfe et al., 1992a).

7.13.5.1. *Comment*

The limited information on the risk of diabetes or other alterations in glucose metabolism in humans in relation to 2,3,7,8-TCDD is not well addressed by the available animal literature, of which there is very little. The effects of 2,3,7,8-TCDD on glucose metabolism have been evaluated only in a few laboratory studies. Although these studies suggest that 2,3,7,8-TCDD may alter glucose metabolism, for the most part the animal studies do not corroborate the direction of the findings of two cross-sectional medical studies (Wolfe et al., 1992a; Sweeney et al., 1992). Animal studies appear to contradict the human data, suggesting that 2,3,7,8-TCDD lowers glucose levels. Several studies of rats and rhesus monkeys showed consistent decreases in serum glucose levels after daily doses administered over 30 days (Zinkl et al., 1973) or after a single dose of 2,3,7,8-TCDD (McConnell et al., 1978a; Gasiewicz et al., 1980; Schiller et al., 1986; Ebner et al., 1988) (Table 7-30). In one study glucose levels continued to drop up to 3 weeks postexposure (Gorski et al., 1990).

Table 7-28. Multiple Linear Regression Model for Log Fasting Serum Glucose for TCP Production Workers and Unexposed Referents

Variable	Parameter estimate	Ratio	p-Value
Intercept[a]	4.2112	0.0548	0.0001
Serum TCDD	0.00008	0.00002	<0.001
Age	0.00288	0.00064	<0.001
Body mass index	0.0066	0.0014	<0.007

Adjusted $R^2 = 0.10$.

[a]Number of observations = 508.

Source: Sweeney et al., 1992.

Table 7-29. Adjusted Relative Risk (RR) for Fasting Serum Glucose Levels, Cases of Diabetes, and Mean 2-Hour Postprandial Glucose Levels by Category of Lipid Adjusted Serum 2,3,7,8-TCDD in Ranch Hands

Category serum 2,3,7,8-TCDD (pg/g)	Fasting serum glucose (RR)	Diabetes (RR)[a]	2-Hour postprandial glucose level (RR)[b]
Unknown ≤ 10[c]	0.66	0.82	0.88
Low 15-≤ 33.3[c]	1.18	1.01	0.60
High >33.3[c]	2.95[d]	2.51[d]	2.35[e]

[a]Defined as having a verified history of diabetes or 2-hour postprandial glucose level of ≥ 11.1 mmol/L (200 mg/dL).
[b]Comparison of diabetics and normals.
[c]Serum 2,3,7,8-TCDD levels in pg/g of lipid.
[d]$p<0.001$.
[e]$p=0.035$.

Source: Roegner et al., 1991.

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Table 7-30. Effects of Exposure to 2,3,7,8-TCDD on Serum Glucose Levels in Nonhuman Mammalian Species

Author	Species	Route	Dose (μ/kg)	Duration	Percentage of serum glucose levels in control animals[a]
Zinkl et al., 1993	CD rat	oral	0.1 1.0 10.1	1x/day for 30 days	91[b] 71 51
Gasiewicz et al., 1980	Rats	ip (TPN) ip (chowfed)	100[c]	1 time	29[d] 51[d]
Schiller et al., 1986	Fischer rat	gavage	30 60 90 180[c] 270[c] 360[c]	1 time	74[d,e] 54[d,e] 30[d,e] 43[d,e] 38[d,e] 39[d,e]
Gorski et al., 1990	Sprague-Dawley rat	ip	125[c]	1 time	day 4 75[b,d] day 8 67[b,d] day 16 50[b,d] day 21 31[b,d]
McConnell et al., 1978a	Rhesus monkey	gavage	70 350[c]	1 time	(decreased)[b,d]
DeCaprio et al., 1986	Hartley guinea pig	oral	0.01 0.06 0.44	90 days	NC[b,e,f] NC[b,e,f] NC[b,e,f]
Ebner et al., 1988	New Zealand rabbits	ip	1 50	1 time	NC 15 min NC 1 hour 125[d,e] 2 day 87 10 day 80[d,e]

[a]Relative to control values.
[b]Female animals.
[c]Lethal dose.
[d]Significantly different from controls.
[e]Male animals.
[f]Data not displayed.
ip = intraperitoneal.
TPN = total parenteral nutrition.
NC = no change.

Dose-related decreases were also noted in CD rats fed 0.1, 1.0, or 10.0 μg/kg daily for 30 days (Zinkl et al., 1973). In contrast, lower daily doses of approximately 0.004 μg/kg/day administered to guinea pigs over a 90-day period produced no significant changes in either glucose or insulin levels (DeCaprio et al., 1986). The results of the animal studies suggest that glucose levels are altered, generally decreased, by short-term, high-dose exposure to 2,3,7,8-TCDD but that the response may be species specific. The diametrically opposed findings of both human and most animal studies may, hypothetically, be due to a number of factors, for example, the species studied, the length of the exposure and short observation periods, the rate of insulin metabolism after 2,3,7,8-TCDD exposure, possible differential effects of 2,3,7,8-TCDD on the various types of islet cells, and the high, usually single, 2,3,7,8-TCDD dose.

The outcome measures used in the NIOSH and the Ranch Hand studies, case of diabetes and fasting serum glucose, did not permit a determination of the type of diabetes involved. However, both studies show that highly 2,3,7,8-TCDD-exposed individuals may be at a slightly greater risk for developing diabetes than individuals with background levels of 2,3,7,8-TCDD. They also experience a greater prevalence of elevated fasting glucose levels, which may be a precursor to conversion to a diabetic state. However, in the NIOSH study, the traditional risk factors for diabetes--age, body mass index or weight, and family history of diabetes--appear substantially more influential than 2,3,7,8-TCDD in the development of diabetes.

7.13.6. Immunologic Effects

Animal toxicologic studies have demonstrated numerous immunologic effects after exposure to 2,3,7,8-TCDD (see Chapter 4 for a more comprehensive review). In humans, the information with which to assess the immunologic consequences of exposure is sparse. Six epidemiologic studies and one case report have described the immunologic function of populations exposed to 2,3,7,8-TCDD (Evans et al., 1988; Hoffman et al., 1986; Webb et al., 1989; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Jennings et al., 1988; Reggiani, 1978; Ott et al., 1993b) and as has one study of extruder personnel exposed to brominated dioxins and furans (Zober et al., 1992).

Evaluation of the immunologic status in exposed residential populations has not found a relationship between exposure and impaired status. Immunocompetence was tested twice in 44 children who were residents of the region of Seveso with the highest 2,3,7,8-TCDD contamination and in 43 age-matched children who did not reside in the contaminated area (Reggiani, 1978). Twenty of the exposed children had chloracne and 24 had no skin lesions. The tests included serum immunoglobulins, complement levels, lymphocyte subpopulations, and lymphocyte activity analysis. Although no data were presented, the authors reported that the various measures were within normal range and that there was no difference between the two groups.

Initial studies of Missouri residents who had the potential for exposure to 2,3,7,8-TCDD-contaminated soil suggested that 2,3,7,8-TCDD caused depression in cell-mediated immunity (delayed hypersensitivity), as demonstrated by a statistically significant increase in anergy (exposed vs. nonexposed: 11.8% vs. 1.1%) (Hoffman et al., 1986). This study, however, was limited by the exclusion from test results of 61% and 32% of the exposed and unexposed groups, respectively. A follow-up study confirmed the presence of substantial bias in the first study. Evans et al. (1988) retested 28 of the 50 exposed residents and 15 of the 27 unexposed residents who did not respond (anergic) or responded weakly (relatively weakly) to an antigen challenge in the first study. A follow-up study could not confirm the presence of anergy. Both studies found in the exposed residents increased frequencies in CD4/CD8 ratio of less than 1.0 (Table 7-31). No other abnormalities were noted by Hoffman et al. (1986) (Tables 7-32 to 7-37).

In a later study of Missouri residents, Webb et al. (1989) found no clinical evidence of immunosuppression in 40 individuals whose adipose 2,3,7,8-TCDD levels ranged from under 20 pg/g to over 430 pg/g (top of range not given). Tests included serum immunoglobulins, T-cell surface markers OKT3, OKT4, OKT8, OKT11, Leu11c, CD4/CD8 ratio, (CD4 + CD8)/CD3, and B1 and B2 cells. In logistic regression, significant ($p < 0.05$) relationships were noted for IgG, %CD3, %T11, %CD8, and %CD4 + LEU8 POS, controlling for age and sex (Tables 7-32 to 7-37).

The effect of past occupational exposure on immunologic function was examined in 18 British workers who were evaluated 17 years after accidental industrial exposure to

Table 7-31. CD4/CD8 Ratios in Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level (SD)	Ratio	N	Mean level (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[a]	126	1.72	---	301	1.89	---
	Low 15-≤33.3[a]	72	1.91				
	High >33.3[a]	72	1.99				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.8[b]	OR<Reference Range 0.9 OR>Reference Range 1.1	1,972	1.8[b]	---
Hoffman et al., 1986	Missouri residents	135	1.9 (0.8)	% Abnormal 8.2	142	1.9 (0.6)	% Abnormal 6.3
Webb et al., 1989	Missouri residents						
	<20[c]	16	2.0 (0.7)	---	---	---	---
	20-60[c]	12	2.1 (1.0)				
	>60[c]	12	1.4 (0.7)				
Ott et al., 1993b	BASF accident cohort	132	1.6 (0.94)	---	42[d]	1.5 (0.6)	---
Zober et al., 1992	BASF personnel exposed to TBDD and TBDF[e]	21	1.6 (0.5)	---	42	1.5 (0.6)	---

[a]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[b]Geometric mean.
[c]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[d]From Zober et al., 1992.
[e]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins and TBDF = 2,3,7,8-tetrabrominated dibenzofurans.

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Table 7-32. Total Lymphocytes in 2,4,5-T Production Workers, Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)[b]	Ratio	N	Mean level (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[c]	127	1,954 ---[d]	---	301	1,972 ---	
	Low 15-≤33.3[c]	73	2,011 ---				
	High >33.3[c]	74	2,032 ---				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,973 ---	OR<Reference Range 1.0 OR>Reference Range 1.2	1,972	1,936 ---	
Hoffman et al., 1986	Missouri residents	135	2,465 (724)	---	142	2,311(634)	
Webb et al., 1989	Missouri residents			% Lymphocytes			
	<20[e]	16	2,200 (830)	32			
	20-60[e]	12	2,300 (600)	32	---	---	
	>60[e]	12	2,200 (720)	28			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	1,980 (840)	---	15	2,020 (470)	

[a]Units: counts/mm^3.
[b]SD = standard deviation.
[c]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[d]--- = data not presented.
[e]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[f]From Zober et al., 1992.
[g]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins; TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-32. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)[b]	Ratio	N	Mean level (SD)	Ratio
Ott et al., 1993b	BASF accident cohort	133	1,978.3 (805)	% Lymphocytes 33.4 (9.4)	42	2,267.6 (837.5)	% Lymphocytes 36 (12.4)
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	2,179.5 (678)	% Lymphocytes 33.4 (8.4)	42	2,267.6 (837.5)	% Lymphocytes 36 (12.4)

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Table 7-33. B1 Levels in Production Workers, 2,4,5-T Missouri Residents, Vietnam Veterans, BASF Accident Cohort, and Extruder Personnel

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[b]	127	176 ---		301	172 ---	
	Low 15-≤33.3[b]	71	183 ---				
	High >33.3[b]	73	191 ---				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	240[c]	OR<Reference Range 1.1 OR>Reference Range 1.2	1,972	230[c] ---	
Webb et al., 1989	Missouri residents			% B1 cells			
	<20[d]	16	190 (865)	9.1	---	---	
	20-60[d]	12	189 (983)	8.3			
	>60[d]	12	171 (573)	7.8			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	210 (110)	---	15	160 (80)	

[a]Units: cells/mm^3.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g lipid.

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Table 7-33. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)	Ratio
Ott et al., 1993b	BASF accident cohort	133	10.4[e] (6.0)	---	42	12.3[e,f] (5.1)	---
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	276.3 (156)	% B cells 12.5 (4.4)	42	286.4 (199.3)	12.3 (5.1)

[e]% B1 cells.
[f]From Zober et al., 1992.
[g]TBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxin
TBDF = 2,3,7,8-tetrabrominated dibenzofuran.

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Table 7-34. CD4 Levels in Production Workers, Missouri Residents, Vietnam Veterans, and BASF Chemical Workers

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[b]	127	867 ---		301	907 ---	---
	Low 15-≤33.3[b]	72	945 ---				
	High >33.3[b]	72	929 ---				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,020[c] ---	OR<Reference Range 1.0 OR>Reference Range 1.4	1,972	990[c] ---	---
Hoffman et al., 1986	Missouri residents	135	1,021 (353)	% Abnormal 0.7	142	1,033 (346)	% Abnormal 0.0
Webb et al., 1989	Missouri residents			% T4 cells			
	<20[d]	16	1,084 (485)	48	---	---	---
	20-60[d]	12	1,198 (391)	51			
	>60[d]	12	963 (403)	42			

[a]Units: cells/mm^3.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.

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Table 7-34. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level (SD)	Ratio
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	950 (340)	---	15	1,040 (290)	---
Ott et al., 1993b	BASF chemical workers	133	---	% T4 cells 42.5 (10.4)	42[e]	---	% T4 cells 45.1 (8.9)
Zober et al., 1992	BASF personnel exposed to TBDD[f] and TBDF[f]	21	973.3 (381.9)	% CD4 44.8 (6.1)	42	1,032 (444.6)	% CD4 45.1 (8.9)

[e]From Zober et al., 1992.
[f]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-35. CD8 Levels in 2,4,5-T Production Workers, Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)	Ratio
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10[b]	126	485 ---		301	473 ---	
	Low 15-≤33.3[b]	71	465 ---				
	High >33.3[b]	73	475 ---				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	560[c] ---	OR < Reference Range 1.0 OR > Reference Range 0.9	1,972	550 ---	
Hoffman et al., 1986	Missouri residents	135	592 (223)	% Abnormal 1.5	142	578 (198)	% Abnormal 0.0
Webb et al., 1989	Missouri residents			% T8 cells			
	<20[d]	16	562 (215)[e]	26			
	20-60[d]	12	645 (225)	28			
	>60[d]	12	807 (381)	35			

[a]Units: cells/mm^3.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose tissue 2,3,7,8-TCDD level in pg/g of lipid.
[e]$p < 0.05$.

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Table 7-35. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)	Ratio
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	630 (280)	---	15	590 (230)	---
Ott et al., 1993b	BASF accident cohort	132	---	% T8 cells 31.9 (10.4)	42[f]	---	% T8 cells 32.0 (7.1)
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	665 (282.6)	% CD8 30.8 (7.9)	42	717.2 (282.4)	% CD8 32.0 (7.1)

[f]From Zober et al., 1992.
[g]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-36. IgG Levels in Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed	
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel					
	Unknown ≤10[b]	335	1,087 ---	---	757	1,120
	Low 15-≤33.3[b]	190	1,122 ---			
	High >33.3[b]	175	1,122 ---			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,078[c]	OR<Reference Range 1.0 OR>Reference Range 1.0	1,972	1,077[c]
Webb et al., 1989	Missouri residents					
	<20[d]	16	1,064[e] (273)	---	---	---
	20-60[d]	12	1,146 (193)			
	>60[d]	12	1,151 (223)			
Ott et al., 1993b	BASF accident cohort	132	1,199[f] (226)	---	194	1,182.6 (310.0)
Zober et al., 1992	BASF personnel exposed to TBDD[g] and TBDF[g]	21	1,057.7 (199.0)	---	42	1,102.9 (207.1)

[a]Units: mg/dl.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[e]$p<0.05$ trend.
[f]Significant positive relation between IgG and current 2,3,7,8-TCDD level and back-extrapolated 2,3,7,8-TCDD level ($p<0.01$).
[g]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-37. IgM Levels in Missouri Residents, Vietnam Veterans, BASF Accident Cohort, and Extruder Personnel

Author	Population	Exposed			Unexposed	
		N	Mean level[a] (SD)	Ratio	N	Mean level[a] (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel				757	103
	Unknown ≤10[b]	335	107 ---			
	Low 15-≤33.3[b]	190	96 ---			
	High >33.3[b]	175	106 ---			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	121[c] ---	OR<Reference Range 1.0 OR>Reference Range 1.0	1,972	121[c]
Webb et al., 1989	Missouri residents					
	<20[d]	16	128 (89)	---	---	---
	20-60[d]	12	157 (57)			
	>60[d]	12	114 (44)			
Ott et al., 1993b	BASF accident cohort	132	139.6 (65.1)	---	192	134.7 (70)
Zober et al., 1992	Personnel exposed to TBDD[e] and TBDF[e]	21	142[f] (52.6)	---	42	114.7[f] (46.5)

[a]Units: mg/dl.
[b]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[c]Geometric mean.
[d]Adipose 2,3,7,8-TCDD level in pg/g of lipid.
[e]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.
[f]p=0.04.

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chemicals contaminated with 2,3,7,8-TCDD (Jennings et al., 1988). It is not clear from the article when occupational exposure to 2,3,7,8-TCDD ended for these workers. Exposed workers and unexposed controls were matched for age, race, sex, smoking habit, alcohol consumption, and percent of ideal body weight. There were no significant differences in the levels of immunoglobulins, T and B lymphocytes, responsiveness to phytohemagglutinin A, and in the number of CD4 and CD8 counts. Three measures were found to be statistically significantly ($p<0.05$) higher in workers than in controls: antinuclear antibodies (ANA) (8 workers vs. 0 controls, $p<0.01$) (when Hep2 cells were used as substrate but not when rat liver cells were used), immune complexes (workers = 11 vs. 3 controls, $p<0.05$), and natural killer cells (NK) (workers = 0.21×10^6/L vs. controls = 0.59×10^6/L, $p<0.002$) identified by the monoclonal antibody Leu-7 (Table 7-38). No other well-conducted studies of exposed individuals have measured these parameters. In the discussion, the authors could not explain the physiologic basis of their findings and suggested that further research was needed.

Among participants in the BASF accident study cohort, with the exception of natural killer cells and helper-inducer cells, the proportions of some lymphocyte populations (B cells, T cells, T helper cells, T suppressor cells) were lower among workers, but the distribution of cells in referents and workers was equivalent (Ott et al., 1993b). Levels for IgA, IgG, IgM, and complement C4 and C3 were slightly higher in workers than in the unexposed referent population. There also appeared to be slight dose-related increases in IgA, IgG, and complement C4 with 2,3,7,8-TCDD levels measured in October 1988 and February 1992. IgA and IgG were related to the half-life extrapolated 2,3,7,8-TCDD levels. It must be noted, however, that the statistically significant relationship between IgA and 2,3,7,8-TCDD is most likely due to a case of liver cirrhosis and the association with IgG due to a liver carcinoma.

Among the 21 extruder personnel exposed to both 2,3,7,8-TBDD and 2,3,7,8-TBDF, of the 16 parameters tested, only complement C4 was statistically significantly ($p<0.01$) associated with concentrations of 2,3,7,8-TBDD and 2,3,7,8-TBDF (Zober et al., 1992). Borderline associations were noted between 2,3,7,8-TBDF and decreases in total lymphocyte count ($p=0.056$), T-cell count ($p=0.045$), T-helper cell count (CD4) ($p=0.045$) and an

Table 7-38. Levels of Natural Killer Cells in Missouri Residents, Vietnam Veterans, and Extruder Personnel

Author	Population	Exposed		Unexposed	
		N	Mean level (SD)	N	Mean level (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel				
	Unknown ≤10[a]	126	455[b,c]	291	414[b]
	Low 15-≤33.3[a]	70	378		
	High >33.3[a]	72	386		
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	400[e] (210)[d]	15	590[e] (230)
Zober et al., 1992	BASF personnel exposed to TBDD[f] and TBDF[f]	21	280.5 (175.2)	42	250.8 (149.9)

[a]Serum 2,3,7,8-TCDD level in pg/g of lipid.
[b]Units: cpm.
[c]Net response.
[d]$p < 0.05$.
[e]Units: $10^3/mm^3$.
[f]TBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;
TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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increase in complement C3 (0.054), and between 2,3,7,8-TBDD and a decreased percent lymphocyte count (p=0.054). However, with the exception of complement C3, the associations appear to be driven by a single individual with the highest 2,3,7,8-TBDD levels (478 pg/g of lipid) who, at the time of the study, exhibited no evidence of clinical immunodeficiency.

Two studies extensively evaluated immunologic function in Vietnam veterans. No significant differences were noted among U.S. Army ground troops and the comparison population in lymphocyte subset populations, T-cell populations, or serum immunoglobulins (Tables 7-31 to 7-37) (Centers for Disease Control Vietnam Experience Study, 1988a). Comprehensive immunologic profiles were developed for each participant of the U.S. Air Force Ranch Hand Study (Tables 7-31 to 7-38) (Roegner et al., 1991). Significant positive associations were found between IgA and serum 2,3,7,8-TCDD. The authors suggest that the rise in IgA is consistent with a subclinical inflammatory response, but the authors could not explain the source of the inflammatory response.

7.13.6.1. *Comment*

The information on immunologic function in humans, children or adults, relative to exposure to 2,3,7,8-TCDD is scarce. The available epidemiologic studies are restricted to adults and do not describe a consistent pattern of effects among the studies. Natural killer cells (NK) were increased among one population of 2,3,7,8-TCDD chemical workers examined 17 years after exposure ended (Jennings et al., 1988). These findings were not corroborated in Ranch Hands (Roegner et al., 1991), the BASF accident cohort (Ott et al., 1993b), or workers exposed to 2,3,7,8-TBDD and TBDF (Zober et al., 1992). Dose-related elevations in IgA were observed in Ranch Hands in relation to current levels and in the BASF accident cohort with respect to both current and half-life extrapolated 2,3,7,8-TCDD levels. Yet, IgA was not higher in adult Missouri residents with adipose 2,3,7,8-TCDD levels above background (Webb et al., 1989). IgG was also significantly related to 2,3,7,8-TCDD in the BASF accident cohort but not in Ranch Hands. While complement C3 and C4 were elevated in both the BASF accident cohort and the extruder personnel, no other study examined these end points.

The effect of acute, high exposure to 2,3,7,8-TCDD among children from Seveso was reportedly negative within 2 years after exposure (Reggiani, 1978). Although no data have been published illustrating the values obtained from the tests of immunologic function in these children, the author indicates that the measured parameters were no different in the exposed and unexposed children after two series of tests.

More comprehensive evaluations of immunologic function with respect to 2,3,7,8-TCDD exposure are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immunologic system may provide the greatest insight, particularly since animal studies have found many of their significant results in immature animals and breast milk is a source of 2,3,7,8-TCDD and other related compounds. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, is a immunotoxin in humans.

7.13.7. Neurologic Effects

Although there are few studies reporting neurologic abnormalities related to 2,3,7,8-TCDD exposure in adult animal models, neurologic effects are reported to have occurred shortly after exposure in occupationally exposed individuals (Ashe and Suskind, 1950; Baader and Bauer, 1951; Bauer et al., 1961; Goldman, 1972; Jirasek et al., 1974; Oliver, 1975; Pocchiari et al., 1979) (Table 7-39) and in Seveso residents (Filippini et al., 1981) (Table 7-40). Previous case reports and studies found symptoms referable to the central (CNS) and peripheral (PNS) nervous system among workers and community residents exposed to 2,3,7,8-TCDD-contaminated materials. While human studies reveal a wide spectrum of effects due to 2,3,7,8-TCDD (Sweeney et al., 1989), very few toxicologic studies have focused on the nervous system. Singer et al. (1982) reviewed the following animal studies, which examined the relationship of CNS dysfunction and 2,3,7,8-TCDD exposure: Elovaara et al. (1977) found anomalous CNS function in some rats exposed to a single dose of 2,3,7,8-TCDD, and Creso et al. (1978) reported the CNS symptoms of irritability, restlessness, and increased aggression in rats administered 2,3,7,8-TCDD. No

Table 7-39. Case Reports of Psychological and Neurologic Effects Among Individuals Exposed to 2,3,7,8-TCDD-Contaminated Materials

Author	Exposure	Population	Evaluation	Findings
Ashe and Suskind, 1950 West Virginia	Precursor and reaction products of TCP process TCP,[a] NaOH,[b] 2,4,5-T[c]	4 employees involved in cleanup after 1949 explosion or worked with equipment used prior to explosion; studied 6 mos and 1 yr after explosion	Clinical history and examination	Headaches Insomnia Social withdrawal Nervousness Fatigue Crying spells Numbness in feet Decreased libido, muscle strength, sensory ability, myelin sheath and fiber destruction of sural nerve
Suskind et al., 1953 West Virginia	TCP 2,4,5-T	36 workers with chloracne after TCP reactor explosion 25 persons exposed during production of TCP and 2,4,5-T 1948-1953	Clinical history and examination	Fatigue Nervousness Irritability Reduced libido Back and limb pain Vertigo Paresthesia
Baader and Bauer, 1951 Nordheim-Westfalan, West Germany	PCP[d] TCP	10 men in PCP production experimental TCP production	Record review case history	Self-reported: pain and weakness Paresthesia and pain in gluteal and femoral region

[a]TCP = 2,4,5-trichlorophenol.
[b]NaOH = sodium hydroxide.
[c]2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.
[d]PCP = pentachlorophenol.

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Bauer et al., 1961 Hamburg, West Germany	TCP 2,4,5-T	31 men in TCP and 2,4,5-T production with residual chloracne, continual "neuromuscular weakness," vasovagal disturbances, psychopathological disturbances" 5 yrs after first exam	Clinical history and examination Hamburg-Wechsler Test Rorschach Psychogram	Reduced libido Headaches, dizziness Decreased libido Irritability Depression Sleep disturbances Anorexia Paresthesia Tremor Muscle weakness Hamburg-Wechsler indicative of acquired decrease in mental efficiency Rorschach Psychogram indicative of decreased emotional reactivity, slowed thinking process for concentration
Goldman, 1972 Ludwigshafen, West Germany	TCP 2,4,5-T	42 BASF workers with chloracne after TCP reactor explosion in 1953	Clinical history and examination	Neurasthenia

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Jirasek et al., 1974 Spolano, Czechoslovakia	TCP 2,4,5-T PCP, HCB[e]	55 workers in TCP and 2,4,5-T production Followed: 1969-1973	Clinical examination history and examination	35 patients with "neurasthenic or depressive syndrome"
Oliver, 1975 England	Pure 2,3,7,8-TCDD	3 scientists synthesis of 2,3,7,8-TCDD	Not described	Subject A: Fatigue and headache Subject B: Headaches Loss of vigor and fatigue Irritability and anger Poor concentration Subject C: Loss of concentration (apathy and fatigue) "Loss of energy and drive" Some difficulty sleeping

[e]HCB = hexachlorobenzene.

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Pazderova-Vejlupkova et al., 1981 Spolano, Czechoslovakia	TCP 2,4,5-T PCP, HCB	4-year follow-up of 44 production workers	Clinical psychiatric examination	Psychiatric changes at start of "intoxication" 83% with neurotic symptoms; neurasthenia syndromes with depressive component; depressive syndrome with endogenous component (N=55) 16% with pseudoneurasthenia syndrome and CNS arteriosclerosis 3% with no psychiatric signs or symptoms Psychiatric changes in exposed individuals at follow-up (1973-1979) 58% with neurotic symptoms and no depressive or anxiety symptoms 18% with severe pseudoneurasthenia and signs of dementia (usually in patients over 50 years old but dementia occurred in 30-year-old patient)

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Pazderova-Vejlupkova et al., 1981 (cont.)				24% with no psychiatric signs or symptoms.
Kimmig and Schultz, 1957b Hamburg, Germany	TCP 2,4,5-T	31 production workers with chloracne (1953-1954)	Clinical history and examination	Tiredness (N=3) Headaches (N=5)
Poland et al., 1971 New Jersey	TCP 2,4,5-T 2,4-D[f] HCB TCP reactor explosion in 1960 Daily exposure 1951-1969	73 male workers in production, maintenance, office areas	Noted histories of smoking, alcohol, medications MMPI[g] given to 52 production and 17 unexposed administrative staff	Headaches (N=8) Severity of acne significantly correlated with high score on the mania scale of MMPI.

[f]2,4-D = 2,4-dichlorophenoxyacetic acid.
[g]Minnesota Multiphasic Personality Inventory.

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Table 7-40. Cross-Sectional Studies of Psychological and Neurologic Effects Among Residents of Missouri and Seveso Exposed to 2,3,7,8-TCDD-Contaminated Materials

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Webb et al., 1989 Missouri	2,3,7,8-TCDD-contaminated waste oil 1971	68 volunteers in State Dioxin Registry; estimated exposure to 20-100 ppb TCDD for 2 yrs or 100 ppb for 6 months	36 volunteers in State Dioxin Registry with no history of exposure to 2,3,7,8-TCDD	Missouri Dioxin Health Studies. Progress Report (1983)	No differences in neurologic exam or in feeling of pins-needles; loss of sensation in extremities, tingling in fingers and toes, diminished VIB 256, diminished VIB 64, diminished pin sensation, diminished thermal sensation (PRR=2%)[a]
Pocchiari et al., 1979 Seveso, Italy	TCP TCP reactor accident July 1976	446 residents of Seveso and Meda, Italy 200 workers from Icmesa plant	255 residents of nearby towns without contamination None	Not detailed but included clinical exam and NCV[b] Exam, EMG[c], NCV	1977: neurologic damage in 6.7% of Seveso residents and in 1.2% unexposed residents 1978: neurologic damage in 11.7% of Seveso residents workers: 4% neuropathic clinical signs exam, EMG, NCV

[a]Prevalence risk ratio.
[b]Nerve conduction velocity.
[c]Electromyography.

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Table 7-40. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Filippini et al., 1981 Seveso, Italy	See description above	308 residents of Seveso, Italy	305 residents of nearby towns without contamination	Symptoms: pain, tingling, numbness Sensory review Muscle tone and strength NCV of ulnar and peroneal nerves	Elevated PRR for peripheral neuropathy in Seveso residents with indicators of exposure (high GGT, ALT, AST, or chloracne) (PRR=2.8, 95% CI=1.2-6.5) Seveso residents with predisposing factors (alcohol or inflammatory disease) (PRR=2.6, 95% CI=1.2-5.6)

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animal studies have specifically evaluated peripheral or autonomic functions related to 2,3,7,8-TCDD exposure.

7.13.7.1. *Neurobehavioral Assessments*

Numerous case reports cite symptoms referable to the nervous system occurring after acute exposure among occupationally exposed individuals (Creso et al., 1978; Ashe and Suskind, 1950; Suskind et al., 1953; Goldman, 1972), as well as chronic exposure to 2,3,7,8-TCDD-contaminated materials (Oliver, 1975; Baader and Bauer, 1951; Kimmig and Schulz, 1957a, b; Bauer et al., 1961; Poland et al., 1971). Symptoms include headache (Ashe and Suskind, 1950; Bauer et al., 1961; Jirasek et al., 1974; Oliver, 1975; Kimmig and Schulz, 1957a, b; Poland et al., 1971), insomnia (Ashe and Suskind, 1950; Suskind et al., 1953; Oliver, 1975; Kimmig and Schulz, 1957a, b), nervousness or irritability (Ashe and Suskind, 1950; Suskind et al., 1953; Bauer et al., 1961; Oliver, 1975), depression and anxiety (Bauer et al., 1961; Jirasek et al., 1974), loss of libido, and encephalopathy (Jirasek et al., 1974; Kimmig and Schulz, 1957a) (Table 7-39).

Some reports indicate that symptoms referable to the CNS and PNS may persist in some exposed individuals for as long as 25 years (Suskind et al., 1953; Poland et al., 1971; Jirasek et al., 1973; Jirasek et al., 1974; Creso et al., 1978; Ashe and Suskind, 1950; Suskind et al., 1953). In 1953, Suskind et al. reported a variety of CNS-related symptoms in 36 workers from a plant in Nitro, West Virginia, who had developed chloracne and other symptoms after exposure to contaminants subsequent to a TCP reactor explosion in March 1949 (N=11) or during normal production process of TCP and 2,4,5-T (N=25) between 1948 and 1953 (Suskind et al., 1953). Such symptoms reported among this relatively young group (average age = 36 years, range = 22-63 years) included fatigue (N=21), nervousness and irritability (N=17), and decreased libido (N=13). No attempt was made to determine whether these symptoms also occurred among exposed individuals without chloracne or among the nonexposed plant population.

Between 1968 and 1969, Jirasek et al. (1974) "observed very closely" (by clinical evaluation) a group of 55 workers exposed to 2,3,7,8-TCDD in the production of 2,4,5-T, hexachlorobenzene, and pentachlorophenol. As described in the report, intoxication with

2,3,7,8-TCDD "occurred gradually from 1965 to 1968" although further quantification of exposure is not described. Psychiatric examination revealed the following: severe neurotic symptoms (64%), neurasthenia syndrome with depressive component (11%), depressive syndromes (8%), pseudoneurasthenia syndromes in patients with arteriosclerosis of the CNS (14%), and normal psychiatric examination (3%). One patient died at age 57 years with rapidly progressive dementia secondary to an atypical arteriosclerosis involving brain and other organs.

Ten years after the initial examination, Pazderova-Vejlupkova et al. (1981) evaluated the health status of 44 of the original 55 workers. They found the following on psychiatric examination: 58% continued to have neurotic symptoms without depressive or anxiety components, 18% developed severe neurasthenia syndromes with signs of dementia, and 24% were normal.

Poland et al. (1971) administered the Minnesota Multiphasic Personality Inventory (MMPI) to 52 male production workers who were exposed at that time to TCP, 2,4,5-T, 2,4-D, and other chemicals. Severity of acne correlated significantly with a high score on the hypomania scale of the MMPI. When production workers were compared with 17 presumably unexposed administrative workers, the two groups differed on only one MMPI symptom scale; exposed production workers scored higher on the hypochondriasis scale.

Table 7-41 describes the results of neurologic and neurobehavioral assessments of TCP production workers, Ranch Hands, and U.S. Army Vietnam veterans.

Moses et al. (1984) found a significant excess among workers with chloracne compared to those without lesions for the following symptoms: insomnia, decreased libido, and difficulties with ejaculation or erection. There was no difference between the two groups for the symptoms of fatigue, irritability, nervousness, depression, or personality changes.

In the study by Suskind and Hertzberg (1984), psychological symptoms were evaluated by interview and peripheral nerve function by nerve conduction velocity of the peroneal motor and sural sensory nerve fibers. The complaint of loss of libido was more frequent among the exposed than unexposed even after stratification by age. The complaint of nervousness, depression, or anxiety was not significantly related to exposure (16.3% vs. 11.7%) in the crude analysis even though the sample size was sufficient to detect a twofold

Table 7-41. Cross-Sectional Studies of Psychological and Neurologic Effects Among TCP Production Workers and Vietnam Veterans Exposed to 2,3,7,8-TCDD-Contaminated Materials

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Moses et al., 1984 West Virginia	TCP[a] 2,4,5-T[b] 1949 TCP reactor explosion	226 workers invited based on union record of employment in production of TCP or 2,4,5-T Definition of exposure = "current or history of chloracne"	Workers without chloracne (N=109) Conduction velocities	Review of symptoms (ROS) obtained by examining physician	Significant diff. with and without chloracne for: insomnia; decreased libido, erection, or ejaculation; no change for fatigue, irritability, nervousness, depression, or personality changes. Decreased pin prick sensation in 18.3% with chloracne; no decreased pin prick sensation without chloracne
Suskind and Hertzberg, 1984 West Virginia	TCP 2,4,5-T	204 workers or maintenance workers in TCP or 2,4,5-T dept.	163 workers never in TCP or 2,4,5-T Mean age of exposed population was younger (56.7 vs. 46.2) ($p<0.0001$).	Interviews and clinical examination	Decreased libido was more frequent among exposed by Mantel-Haenszel Chi by age. Nervousness/depression/ anxiety not increased in exposed (sample size sufficient to detect a 2-fold increase)

[a]TCP = 2,4,5-trichlorophenol.
[b]2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

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Table 7-41. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Sweeney et al., 1993 Alderfer et al., 1992 Missouri and New Jersey	TCP 2,4,5-T	280 production workers with daily exposure New Jersey 1951-1969 Missouri 1968-1972	261 unexposed community residents matched for age, race, gender	Symptom and medical history; neurologic examination; neurophysiologic tests of vibration and thermal sensitivity; Beck Depression Scale; SCL-90-R	No significant difference for symptoms or any examination variable No significant difference in mood disorders
Lathrop et al., 1984	Vietnam service 1962-1971	1,208 Ranch Hands assigned to aerial spraying herbicides and insecticides Republic of Vietnam	1,238 men who flew cargo missions in Southeast Asia Matched by month of birth, race, and occupational code	Self-report of psychological or emotional illness Diagnostic Interview Schedule (modified) Self-reported depression	No difference Significantly more fatigue, anger, erosion, anxiety, closest for high school-educated Ranch Hands; no difference for college-educated Greater for Ranch Hands

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Table 7-41. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Lathrop et al., 1984 (cont.)				Cornell Index (self-administered inventory of neuropsychiatric symptoms) (psychophysiologic)	After adjustment for education 4/10 parameters abnormal (nervousness, anxiety, startle, psychosomatic, gastrointestinal system); abnormal parameters inversely related to education level.
				MMPI	High school-educated Ranch Hands showed significant deficits on scales for hypochondria, masculinity/femininity; mania/hypomania but comparisons show more denial; MMPI scores influenced by education ($p<0.01$)
				Halstead-Reitan Battery	No impairment in Ranch Hands
				Wechsler Adult Intelligence Scale (WAIS)	Scores related to educational level

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Table 7-41. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Roegner et al., 1991	Vietnam service	720 USAF Ranch Hands; serum 2,3,7,8-TCDD levels were measured (lipid adjusted)	779 who flew cargo missions in Southeast Asia; serum 2,3,7,8-TCDD levels were measured	Self-report	No significant mental, emotional, or sleep disorders
				SCL-90-R[a]	No significant differences with increasing serum levels
				Cornell Medical Index (CMI)	Significantly higher mean schizoid and schizotypal scores in Ranch Hands over 33.3 pg/g 2,3,7,8-TCDD, but they did not relate to similar scales in the SCL-90-R
				Neurologic exam	Overall, no consistent relationship between neurologic abnormalities and TCDD level.

[a]SCL-90-R: Symptom Checklist Revised.

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increase. However, in the group over 50 years old there was a significant exposure effect (19% vs. 6.4%) for the complaint of nervousness, depression, or anxiety. The complaint of impotence was significantly related to exposure in the crude analysis, but after stratification by age this effect disappeared.

The effects of exposure to 2,3,7,8-TCDD on measures of current symptoms of depression were evaluated (Alderfer et al., 1992) as part of the NIOSH cross-sectional medical study (Sweeney et al., 1989). Symptoms of depressed mood were measured by the Beck Depression Inventory and the depression subscale of the Self-Report Symptom Checklist-90-Revised (SCL-90-R). Neither serum 2,3,7,8-TCDD levels nor status as a worker was associated with depressed mood as assessed by either the Beck Depression Inventory or the SCL-90-R depression subscale (Alderfer et al., 1992). This finding supports the conclusion that current serum levels of 2,3,7,8-TCDD are not associated with current depression among a population of workers that was highly exposed to TCDD. However, because this cross-sectional study was conducted many years after 2,3,7,8-TCDD exposure, this analysis could not address the question of whether 2,3,7,8-TCDD is associated with past depression that resolved before the study was performed. These findings are consistent with those of the U.S. Air Force study (Roegner et al., 1991) of personnel who applied Agent Orange during the Vietnam war: serum 2,3,7,8-TCDD was not associated with the depression subscale score of the SCL-90-R after controlling for covariates.

The Air Force study conducted neurologic and psychological assessments of the participating Ranch Hands and comparisons (Lathrop et al., 1984). In the first examination series published in 1984 (baseline), the psychological assessment included a self-report of psychological or emotional illness, the Diagnostic Interview Schedule (DIS), Cornell Medical Index (inventory of psychophysiologic symptoms), MMPI, Halstead-Reitan Battery (HRB), and Wechsler Adult Intelligence Scale (WAIS). In the 1987 follow-up examination, the psychological assessment included an interviewer-administered questionnaire in which each participant was asked about the occurrence of mental or emotional disorders and sleep disorders. The presence of posttraumatic stress disorder was based on a subset of questions from the MMPI; the WAIS IQ assessment was deleted and the Millon Clinical Multiaxial

Inventory (MCMI) and the Symptom Checklist-90-Revised (SCL-90-R) were added (Lathrop et al., 1987).

Results of the 1984 baseline study revealed no difference between Ranch Hands and the comparison group for self-reported psychological or emotional illness. The DIS revealed significantly more fatigue, anger, erosion, and anxiety for high school-educated but not college-educated Ranch Hands. These outcomes were highly related to education. The Cornell Medical Index found 4 of 10 parameters abnormal for Ranch Hands--startle, psychosomatic, gastrointestinal nervousness, and anxiety. These parameters were inversely related to education level. On the MMPI, high school-educated Ranch Hands showed significant differences (more deficits) on subscales for hypochondria, masculinity/femininity, and mania/hypomania, but comparisons scored higher on the subscale for denial, a finding that might undermine the deficits noted for Ranch Hands. Again, MMPI scores were influenced by education level ($p<0.01$) but not exposure level. For both the HRB and WAIS, there was no difference between Ranch Hands and comparisons, and the scores were related to education level.

In the 1987 reanalysis with serum 2,3,7,8-TCDD (Roegner et al., 1991), there was no significant difference between groups or relationship with serum 2,3,7,8-TCDD levels on reported (and verified) data on lifetime psychological illness or sleep disorders or any SCL-90-R. Some of the MCMI parameters appeared to be related to serum 2,3,7,8-TCDD levels (significantly higher mean schizoid and schizotypal scores and significantly lower mean histrionic score in the group above 33.3 pg/g than in comparisons). However, these findings were inconsistent with similar variables in the SCL-90-R and the self-reported histories.

Comprehensive neurologic and psychological assessments were conducted on participants of the Vietnam Experience Study (Centers for Disease Control Vietnam Experience Study, 1988a, b). The neurobehavioral tests evaluated aptitude, concept formation and problem-solving, memory, manual dexterity, verbal skills, visuo-motor skills, attention, and mental control. Among Vietnam veterans there was a significantly greater prevalence of alcohol abuse or dependence (Vietnam veterans, 13.7%; non-Vietnam veterans, 9.2%; OR=1.5, 95% CI=1.2-1.8), depression (Vietnam veterans, 4.5%; non-Vietnam veterans, 3.2%; OR=2.0, 95% CI=1.4-2.9), and a higher prevalence of poor psychological

status (Centers for Disease Control Vietnam Experience Study, 1988b). The poor psychological status tended to be most prevalent in Vietnam veterans who were not white, who enlisted before their 19th birthday, and whose enlistment test scores fell below the group median.

In the study of residents of the Quail Run Mobile Home Park, neurobehavioral tests evaluated reaction time, mood, memory, visuo-motor coordination, intelligence, and indicators of psychological stress (Hoffman et al., 1986). Differences between Quail Run residents and controls were observed in the vocabulary subtest of the WAIS (Quail Run = 34.7, controls = 41.1, raw scores; $p<0.01$), tension/anxiety raw score (Quail Run = 13.7, controls = 11.1; $p<0.01$), and anger/hostility scale (Quail Run = 11.6, controls = 8.9; $p<0.05$) of the POMS inventory, and for the depression/dejection and fatigue/inertia scales (no data provided).

7.13.7.2. *Neurologic Status*

On neurologic examination, 11 of 60 West Virginia workers with chloracne exhibited decreased sensitivity to pin prick, whereas none of the 34 subjects without chloracne had decreased pin prick sensation ($p<0.01$) (Moses et al., 1984). There were no other differences in performance of the neurologic examination noted in the text. When examined by Suskind and Hertzberg (1984), no significant differences were noted in the conduction velocities of either nerve fiber (sural sensory: exposed workers, mean = 42.06 ± 0.49; unexposed workers, mean 41.49 ± 0.54; peroneal motor: exposed workers, mean = 41.77 ± 0.47; unexposed workers, mean = 42.62 ± 0.52).

Among New Jersey and Missouri TCP workers, the overall neurologic status and peripheral nerve function were assessed for all 281 workers and 260 referents by self-reported medical history, neurologic examination, electrophysiologic tests of nerve conduction velocity, amplitude and latency, and vibratory and thermal threshold (Sweeney et al., 1993). No differences in neurologic status or nerve function between workers or referents were detected. Additionally, although the mean serum 2,3,7,8-TCDD level in the workers was 220 pg/g, there was no relationship between neurologic function and levels of serum 2,3,7,8-TCDD.

The neurologic examination in the 1987 follow-up study evaluated cranial, CNS, and PNS function in participating Ranch Hands (Lathrop et al., 1987). In general, there was no difference in the prevalence of neurologic abnormalities in Ranch Hands and comparisons. However, Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g tended to have a higher proportion of individuals with abnormal coordination than the comparisons (Ranch Hands, 2.7%; comparisons, 0.4%; adjusted RR=18.3, $p<0.001$) (Roegner et al., 1991).

Overall neurologic status of Army Vietnam veterans did not differ from that of non-Vietnam veterans (Vietnam veterans, 1.0%; non-Vietnam veterans, 0.8%; OR=1.2, 95% CI=0.6-2.3) (Centers for Disease Control Vietnam Experience Study, 1988b). Only self-reported symptoms related to nerve disorders were significantly more prevalent among Vietnam veterans than non-Vietnam veterans (Vietnam veterans, 8.2%; non-Vietnam veterans, 6.5%; OR=1.2, 95% CI=1.0-1.6).

Table 7-40 describes the results of neurologic and neurobehavioral studies of Seveso and Missouri residents. While three studies evaluated the neurologic status of residents of Seveso (Pocchiari et al., 1979; Filippini et al., 1981; Assennato et al., 1989), no studies evaluated neurobehavioral changes. In an effort to quantify exposure-related neurologic disorders among Seveso residents and among workers from the Icmesa plant, two government-sponsored screenings were conducted in 1977 and 1978 on 308 residents of Seveso and 200 workers. Among these workers, approximately 4% (N=8) were found to have damage to nerve fibers of multiple (unspecified) nerves, controlling for confounding factors such as alcohol abuse, diabetes, kidney disease, and neurotoxic medication use (Pocchiari et al., 1979). The report did not describe the extent of worker exposure to 2,3,7,8-TCDD. Other potential neurotoxic occupational exposures do not appear to have been considered. Three workers were described as having polyneuropathies of the lower limbs.

In 1981, prevalence risk ratios (PRR) for neuropathy were calculated separately for the 308 Seveso residents (Filippini et al., 1981). PRRs for neuropathy were determined for residents who exhibited clinical indication of 2,3,7,8-TCDD exposure, defined as the presence of elevated liver enzyme levels (GGT, ALT, AST) (which are also indicative of nonspecific insults to the liver) or chloracne, and for those who exhibited conditions that are

risk factors for neuropathy, e.g., alcoholism, inflammatory disease, diabetes, or potential occupational exposure to neurotoxins. Seveso residents who had clinical indication of 2,3,7,8-TCDD exposure (chloracne, or elevated liver enzymes GGT, AST, ALT) or who had risk factors for neuropathy were found to have significantly greater prevalence of neuropathy than residents without either manifestation (PRR exposure = 2.8, 95% CI=1.2-6.5; PRR for possible 2,3,7,8-TCDD-predisposing factors = 2.6, 95% CI=1.2-5.6) (Filippini et al., 1981). Additional analysis identified that individuals who met the definition for chloracne or abnormal levels of hepatic enzymes were significantly more at risk than residents without either condition.

Residents of Seveso who developed chloracne after the reactor release (N=193) were invited to a series of three follow-up screenings in 1982-1983, 1983-1984, and 1985 (Assennato et al., 1989). A control group from a nearby but uncontaminated area was also examined. Conduction velocities of the median motor, peroneal motor, and sural sensory fibers were conducted in 1982-1983 and 1985 (Assennato et al., 1989). No increases in the prevalence of abnormal electrophysiologic measures were observed in the chloracne group when compared with controls without chloracne. In addition, there was no change in the conduction velocities for each fiber from the 1982-1983 to 1985 studies.

Quail Run residents reported significantly more "numbness" or "pins and needles" in the hands or feet (28.6%) than the controls (18.1%) ($p<0.05$), but there were no differences in mean threshold scores for the more objective neurosensory tests. Residents also reported more persistent severe headaches (Quail Run, 26.0%; control, 14.2%; $p<0.05$).

Participants in the study by Webb et al. (1989) did not complete neurobehavioral tests, but they were examined by a neurologist. The results were unremarkable. Of the 38 participants, two with levels above background had abnormal pin prick sensitivity ($\leq$20 pg/g, N=2; 20-60 pg/g, N=1; $\geq$60 pg/g, N=1), three had abnormal vibration thresholds ($\leq$20 pg/g, N=2; 20-60 pg/g, N=1; $\geq$60 pg/g, N=2), and four had abnormal reflexes ($\leq$20 pg/g, N=2; 20-60 pg/g, N=2; $\geq$60 pg/g, N=2). The results of other components of the neurologic examination were not reported and are assumed to be normal.

7.13.7.3. *Comment*

The overall results of these case reports and epidemiologic studies demonstrate that exposure to 2,3,7,8-TCDD-contaminated materials is associated with symptoms referable to the central and peripheral nervous systems shortly following exposure and, in some cases, lasting many years (Tables 7-40 and 7-41). Symptoms include fatigue, nervousness, anxiety, and decreased libido (Ashe and Suskind, 1950; Suskind et al., 1953; Oliver, 1975; Kimmig and Schulz, 1957a, b; Bauer et al., 1961; Jirasek et al., 1974). One case report found mania/hypomania on the MMPI (Poland et al., 1971). These symptoms are consistent with mood disorder. While one study reported neurasthenia with signs of dementia lasting 10 or more years following exposure (Jirasek et al., 1974), the U.S. Air Force study of Vietnam veterans used neurobehavioral testing but was unable to demonstrate cognitive or other functional CNS deficits. However, this negative study did not investigate the relationship between serum 2,3,7,8-TCDD levels and neurobehavioral deficits. NIOSH investigated measures of depressed mood many years after exposure to 2,3,7,8-TCDD among production workers and found no relationship between depressive symptoms and serum 2,3,7,8-TCDD levels (Alderfer et al., 1992).

Overall neurologic status of workers, community residents, and Vietnam veterans exposed to 2,3,7,8-TCDD and evaluated from 5 to 37 years after last exposure appears to be normal. These data suggest that, although exposure to 2,3,7,8-TCDD may have been extensive as in the case of the exposed workers, Ranch Hands, and Seveso residents and case reports describe many related symptoms, the effects may have been transient. If so, studies conducted years after the last exposure would not detect such changes. These results suggest that, in adults, no long-term neurologic effects were caused by even high exposure to 2,3,7,8-TCDD-contaminated materials. However, there is very little information with which to examine the effects of exposure on the developing human neurologic system.

7.13.8. Circulatory System

The relationship between human exposure to 2,3,7,8-TCDD-contaminated chemicals and disorders of the circulatory system has been explored in a variety of reports. A number of early case reports have described effects on the cardiovascular system among individuals

reportedly exposed to chemicals contaminated with 2,3,7,8-TCDD. Myocarditis (Goldman, 1972), myocardial infarctions (Walker and Martin, 1979; Bauer et al., 1961), ectasia of the coronary arteries (England, 1981), and rapidly progressive atherosclerosis (Jirasek et al., 1974; Pazderova-Vejlupkova et al., 1981) have been reported.

Studies have described mortality from diseases affecting the circulatory system among populations exposed to 2,3,7,8-TCDD (Bond et al., 1987; Coggon et al., 1991; Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993) (Table 7-42). The circulatory system includes ICD-9 codes 390-459 (International Classification of Diseases 9). The results are limited because the studies were primarily designed to test hypotheses relating to cancer and, secondarily, to characterize mortality from causes other than cancer, without detailed consideration of confounders or extent of exposure to 2,3,7,8-TCDD. In addition, because the study populations have differing age distributions, direct comparison of the SMRs is not valid. However, any trends suggested by the direction and magnitude of the SMRs are important.

Among TCP production workers, mortality from all diseases of the circulatory system was similar to mortality in the general population, as described by an SMR of 100, and in workers from The Netherlands (Plant A) (SMR=98, 95% CI=65-142) (Bueno de Mesquita et al., 1993), the United States (Nitro, West Virginia) (SMR=90, 95% CI=80-100) (Collins et al., 1993), and Great Britain (SMR=116, 95% CI=91-146) (Coggon et al., 1991) (Table 7-42). In two studies, mortality in workers with chloracne was not significantly different from that of the national comparison group (U.S. workers, SMR=95, 95% CI=79-113; German workers, SMR=121, 90% CI=83-170) (Bond et al., 1987; Zober et al., 1990).

Mortality from more specific end points, such as ischemic heart disease, all heart disease, and cerebrovascular disease, was noted in a few studies. The SMR from ischemic heart disease was 102 (95% CI=63-158) among Dutch workers (Bueno de Mesquita et al., 1993) and 96 (95% CI=51-164) in U.S. workers (Midland, MI) (Bond et al., 1987). And, in an analysis that included all diseases of the heart combined among 5,200 U.S. production workers, the SMR was 96 (95% CI=87-106) (Fingerhut et al., 1991b). Cerebrovascular disease mortality was slightly elevated among Dutch TCP production workers (SMR=117, 95% CI=38-274) (Bueno de Mesquita et al., 1993) and increased by more than twofold in

Table 7-42. Mortality from Diseases of the Circulatory System in Populations Exposed to 2,3,7,8-TCDD

Author	Population	Outcome	No. of deaths	SMR[a]	95% CI	Cohort size	Years of follow-up
Fingerhut et al., 1991b	2,4,5-TCP and 2,4,5-T production workers, USA	Diseases of the heart (ICD 390-398, 402-404, 410-414, 420-429)	393	96	87-106	5,172	1942-1987
Fingerhut et al., 1991b	2,4,5-TCP and 2,4,5-T production workers, USA	Diseases of the circulatory system (ICD 401, 403, 405, 415-417, 430-438, 440-459)	67	77	60-98	5,172	1942-1987
Zober et al., 1990	2,4,5-T production workers with chloracne, Germany	Diseases of the circulatory system (ICD 390-458)		121	83-170[b]	127	1953-1987
Coggon et al., 1991	2,4,5-T synthesis or formulation, Great Britain	Diseases of the circulatory system	74	116	91-146	2,239	1975-1987
Bond et al., 1987	U.S. chemical production workers: TCP + 2,4,5-T with chloracne (Michigan)	Diseases of the circulatory system ASHD[c] Vascular lesions of CNS	19 13 4	102 96 208	61-159 51-146 57-539	322	1940-1982
Bond et al., 1987	U.S. chemical production: workers without chloracne (TCP + 2,4,5-T) (Michigan)	Diseases of the circulatory system ASHD[c] Vascular lesions of CNS	130 106 10	95 110 64	79-113 90-133 31-117	2,026	1940-1982

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Table 7-42. (continued)

Author	Population	Outcome	No. of deaths	SMR[a]	95% CI	Cohort size	Years of follow-up
Bueno de Mesquita et al., 1993	2,4,5-TCP and 2,4,5-T production workers, The Netherlands	Diseases of the circulatory system (ICD 390-458)	28	98	65-142	549	1955-1985
Bueno de Mesquita et al., 1993	2,4,5-TCP and 2,4,5-T production workers, The Netherlands	Ischemic heart disease (ICD 410-414)	20	102	63-158	549	1955-1985
Bueno de Mesquita et al., 1993	2,4,5-TCP and 2,4,5-T production workers, The Netherlands	Cerebrovascular disease (ICD 430-438)	5	117	38-274	549	1955-1985
Michalek et al., 1990	U.S. Air Force Ranch Hand personnel	Diseases of the circulatory system	25	110	60-150	1,261	1961-1987
Centers for Disease Control Vietnam Experience Study, 1988c	U.S. Army Vietnam veterans	Diseases of the circulatory system (ICD 390-459)	12	0.49[d]	0.25-0.99	9,324	1965-1983
Fett et al., 1987b	Australian Vietnam veterans; served >12 months	Diseases of the circulatory system	20[d]	1.7	0.9-3.0	Vietnam veterans = 19,205 Non-Vietnam veterans = 25,677	1966-1985

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Table 7-42. (continued)

Author	Population	Outcome	No. of deaths	SMR[a]	95% CI	Cohort size	Years of follow-up
Bertazzi et al., 1989	Residents of Seveso, Italy, ages 20-74, Zone A (high TCDD region)	Diseases of the circulatory system (ICD 390-459) ISHD[f] Cerebrovascular disease	11 6 2 5	**Males:** 1.75[d] **Females:** 1.89[d] **Males:** 1.25[d] **Males:** 3.3[d]	1.0-3.2 0.8-4.2 0.5-3.3 1.4-8.0	556[e]	1976-1986
Bertazzi et al., 1992	Residents of Seveso, Italy, ages 1-19 years	Diseases of the circulatory system (ICD 390-459)	0 2	**Males:** not reported **Females:** 1.63[d]	--- 0.3-8.1	306[e]	1976-1986

[a]SMR = Standardized mortality ratio.
[b]90% confidence interval.
[c]Atherosclerotic heart disease.
[d]Relative risk.
[e]Zone A males and females combined.
[f]Ischemic heart disease.

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Michigan TCP production workers with chloracne (SMR=208, 95% CI=57-539) (Bond et al., 1987).

The SMRs for circulatory system diseases reported in the various mortality studies are close to 100, suggesting that the "healthy worker effect" is not seen in these studies. Generally, because employed workers are healthier than the general population, the SMR for cardiovascular disease in employed populations tends to be lower than 100 (McMichael, 1976; Fox and Collier, 1976). The absence of a healthy worker effect, in light of the positive animal data, suggests that more detailed analyses should be conducted for cardiovascular outcomes in these populations.

Among 1,261 Ranch Hand personnel, mortality from circulatory disease was nonsignificantly elevated (SMR=110, 95% CI=60-150) compared with that of a comparison population of 19,101 other Air Force veterans who were not exposed to herbicides (Michalek et al., 1990). Similar nonsignificant increases in the relative mortality ratio (RMR) were observed for circulatory diseases (RMR=1.6, 95% CI=0.8-3.2) in Australian Vietnam veterans (N=19,205; 260 deaths) compared to 25,677 (263 deaths) non-Vietnam veterans who only served in Australia (Fett et al., 1987b). In contrast, the unadjusted relative risk of 0.49 (95% CI=0.25-0.99) for all circulatory diseases suggested a deficit of deaths from this cause among 9,325 Vietnam Army veterans (246 deaths) compared to 8,989 non-Vietnam veterans (200 deaths) (Centers for Disease Control Vietnam Experience Study, 1988c).

Bertazzi and colleagues examined the mortality experience of Seveso residents ages 1-19 years (Bertazzi et al., 1992) and ages 20-74 years (Bertazzi et al., 1989) 10 years after the contamination of the town by 2,3,7,8-TCDD-contaminated effluent. In the younger population, two deaths from circulatory diseases occurred only in female residents (RR=1.63, 95% CI=0.3-8.1). In the older population, circulatory disease mortality of residents from Zone A (the most highly contaminated region) was elevated in both males (RR=1.75, 95% CI=1.0-3.2) and females (RR=1.89, 95% CI=0.8-4.2). In males, the highest death rate occurred during the first quinquennium, 1976-1981 (RR=2.04, 95% CI=1.0-4.2), and, in females, the highest death rate occurred during the second quinquennium, 1982-1986. The authors suggest that the study was limited by the small number of subjects and the crude measure of 2,3,7,8-TCDD exposure. The authors could

not attribute the increased mortality from circulatory disease to 2,3,7,8-TCDD exposure but suggested that the "high stress and pollution" imposed on the residents of Zone A may have been a contributing factor.

Several cross-sectional medical studies have also examined the association between 2,3,7,8-TCDD exposure and effects on the cardiovascular system (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1983; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991). Statistically significant associations relative to 2,3,7,8-TCDD exposure were found only in the Ranch Hand study for diastolic blood pressure, arrhythmias detected on the electrocardiogram (ECG), and peripheral pulse abnormalities (Roegner et al., 1991). However, there is some doubt that the significant findings are dose related because significant increases in the mean diastolic blood pressure were found in Ranch Hands with serum 2,3,7,8-TCDD levels from 15 to 33.3 pg/g but not in the Ranch Hands with higher serum 2,3,7,8-TCDD levels. The adjusted odds ratios for ECG-diagnosed arrhythmias among Ranch Hands were not entirely consistent with a dose-response relationship. For each serum 2,3,7,8-TCDD category the odds ratios were: serum 2,3,7,8-TCDD levels $\leq$10 pg/g, OR=1.33 (95% CI=0.68-2.58, p=0.40); 15-33.3 pg/g, OR=0.83 (95% CI=0.31, 2.23, p=0.71); and for serum 2,3,7,8-TCDD levels above 33.3 pg/g the OR was 2.34 (95% CI=1.00-5.51, p=0.051). The proportion of individuals in this group with arrhythmias (5.2%) was not much higher than in Ranch Hands whose serum levels were $\leq$10 pg/g (4.7%). Finally, relative to the comparison group, the proportion of abnormal peripheral pulses in all Ranch Hands, regardless of serum level, was elevated.

No excess abnormalities or disorders of the circulatory system or heart were found in several groups of TCP production workers, although their potential for exposure to 2,3,7,8-TCDD-contaminated chemicals was high (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1983). The prevalence of hypertension or coronary artery disease (both self-reported), abnormal ECG findings, atherosclerotic changes (not specified) on chest X-ray, or blood pressure elevation was not elevated in West Virginia TCP production workers (Suskind and Hertzberg, 1984). Similarly, when Moses et al. (1984) examined TCP production workers with chloracne and compared them with workers not affected by chloracne, they found no increased risk for self-reported abnormal ECG, self-reported angina, or self-

reported myocardial infarction and no difference in the physical examination of the cardiovascular system. Finally, Bond et al. (1983) found no increased risk for self-reported hypertension among workers involved in the production of trichlorophenol and 2,4,5-T.

7.13.8.1. ***Comment***

The picture relating exposure to 2,3,7,8-TCDD and diseases of the circulatory system is mixed. Animal data indicate that high doses of 2,3,7,8-TCDD affect cardiac and vascular integrity (Allen and Carstens, 1967; Allen et al., 1977; Norback and Allen, 1973). Data from a few animal studies suggest that relatively high doses of 2,3,7,8-TCDD cause damage to the myocardium and heart valves in rats (Kociba et al., 1978; Buu-Hoï, 1972) and to the arterial walls in rabbits (Brewster et al., 1987). Other research found that 2,3,7,8-TCDD may alter cardiac function in rats and guinea pigs (Hermansky et al., 1988; Kelling et al., 1987; Canga et al., 1988), causing reduced spontaneous and isoproterenol-induced heart contractility; this suggests that 2,3,7,8-TCDD may increase the risk of arrhythmias among the dosed animals. The authors postulate that 2,3,7,8-TCDD alters cyclic-AMP concentrations, altering the responsiveness of cardiac cells to β-adrenergic stimuli (Brewster et al., 1987). In contrast, histopathologic changes were not observed in the cardiovascular system of hamsters, which appears to be resistant to the effects of 2,3,7,8-TCDD at levels of 3,000 μg/kg of 2,3,7,8-TCDD (Olson et al., 1980). Other experimental studies suggest an association between 2,3,7,8-TCDD and alterations in lipid levels (Poli et al., 1980; Albro et al., 1978; Bombick et al., 1984; Swift et al., 1981).

Findings from mortality and morbidity studies of production workers are not definitive, and suggest the need for analyses more specifically examining possible effects on the circulatory system and heart. The outcomes examined in the animal and human studies are different. Animal studies describe morphologic and chemical changes in the vascular and cardiac cells caused by 2,3,7,8-TCDD. On the other hand, the diseases and causes of death in the human studies assessed the possible consequences of exposure on long-term pathologic changes to the tissues, which cause cell and, sometimes, organ and system failure. Using the animal data, it is possible to project the long-term consequences of exposure to the organ or system. However, the animal studies do not account for the possibility of intervening events,

such as the repair of tissue after exposure ends, or other events that reduce the hypothesized end point to a much less drastic outcome.

Circulatory diseases and diseases of the heart have not been rigorously investigated as hypothesized health outcomes of exposure to 2,3,7,8-TCDD. Therefore, mortality studies have not considered the possible confounding effect of other variables, including smoking, lipid levels, and other conditions, that influence circulatory diseases and disorders of the heart. Furthermore, the mortality studies tended to examine the circulatory system as a whole, without consideration of the possible pathophysiology of 2,3,7,8-TCDD exposure on the various components of the system, e.g., vessels versus cardiac muscle damage. Finally, mortality studies count only those conditions that ultimately caused the death of the individual. Events such as myocardial infarctions, which may debilitate the individual but not cause death, may be missed if death is caused by another circumstance. Therefore, the effect of 2,3,7,8-TCDD on the coronary arteries might be missed because it was not coded as the underlying cause of death on the death certificate.

With the exception of the Ranch Hand study (Roegner et al., 1991), cross-sectional analyses of other more highly exposed groups were limited by their lack of good exposure data and their inability to examine the relationship between serum 2,3,7,8-TCDD levels and diseases of the circulatory system or heart. Such studies may also be limited by the fact that they include a survivor population, as described in the introduction.

Further research would be useful to define the relationship between the pathologic end points observed in animals after high, single doses of 2,3,7,8-TCDD and the disease outcomes observed in humans after high, long-term exposure. To identify whether 2,3,7,8-TCDD has an effect on the human vasculature, additional work is needed to determine whether certain doses of 2,3,7,8-TCDD cause changes in the human vascular system; to determine whether there are changes in the action of chemicals associated with human cardiac muscle contraction caused by 2,3,7,8-TCDD exposure; and to assess mortality and morbidity in individuals with potential for 2,3,7,8-TCDD exposure while carefully controlling for other risk factors and using more accurate measures of exposure.

7.13.9. Pulmonary Effects

Studies of long-term exposure to 2,3,7,8-TCDD in Sprague-Dawley rats (Kociba et al., 1979; Van Miller et al., 1977), B6C3F1 mice (NTP, 1982a), Swiss-Webster mice (NTP, 1982b), and rhesus monkeys (Allen et al., 1977) have reported changes in bronchiolar or alveolar tissue ranging from epithelial hyperplasia and metaplasia to squamous cell carcinomas. The hyperplastic and metaplastic changes observed in exposed animals are similar to the pathologic picture of chronic bronchitis in humans (American Thoracic Society, 1962).

Case reports have described temporary respiratory irritation (Zack and Suskind, 1980) and tracheobronchitis (Goldman, 1972) among chemical workers exposed to 2,3,7,8-TCDD-contaminated herbicides following industrial accidents. In addition, Baader and Bauer (1951) reported chronic bronchitis in seven workers involved in pentachlorophenol production, which resolved in all but two workers within 2 weeks after production was discontinued.

There is conflicting evidence from controlled epidemiologic studies regarding an association between chronic respiratory system effects and human exposure to substances contaminated with 2,3,7,8-TCDD. One study of workers involved in the production of TCP and 2,4,5-T suggested that 2,3,7,8-TCDD exposure increases the risk for abnormal ventilatory function (Suskind and Hertzberg, 1984). This study found a statistically significantly increased risk for an abnormal forced expiratory volume at 1 second (FEV_1) ($p<0.01$), an abnormal forced vital capacity (FVC) ($p<0.001$), and an abnormal FEV_1/FVC ratio ($p<0.05$) among workers who were smoking at the time of the study. For workers, the percent predicted spirometric parameters for FEV_1, FVC, and FEV_1/FVC were 99.4%, 92.7%, and 76.5% and for referents, 104.4%, 97.6%, and 79.9%, respectively. The only other study of TCP and 2,4,5-T production workers that reported ventilatory function findings found no association between serum 2,3,7,8-TCDD levels and declines in ventilatory function (Calvert et al., 1991). The disparity in results between the two studies may be related to the age of the unexposed populations and potential exposures of the exposed. In the Suskind and Hertzberg study, the exposed workers were, on average, 10 years older than the unexposed workers. Although the authors indirectly adjusted for age by analyzing age-adjusted ventilatory measures, it is not clear if these adjustments can completely control

for a 10-year difference in age. In the study by Calvert et al. (1991), the difference in mean age between the exposed and unexposed groups was 0.6 years. The second difference involves the potential for exposure to 2,4,5-T acid dust at the plants studied. The 2,4,5-T acid that was produced at the plant studied by Suskind and Hertzberg was finished as a powder. At the plants studied by Calvert et al. (1991), the 2,4,5-T acid was finished as a liquid. Therefore, the potential for exposure to 2,4,5-T acid dust was greater at the plant studied by Suskind and Hertzberg (1984). Although we are not aware of any published reports supporting an association between ventilatory function and 2,4,5-T acid exposure, a respiratory burden of particles, in the absence of a specific toxic agent, can be a probable cause of ventilatory function declines (Becklake, 1985).

The Ranch Hand study also examined the association between serum 2,3,7,8-TCDD level and respiratory system effects (Roegner et al., 1991). This study found significant declines in the mean FEV_1 and the mean forced expiratory volume (FVC) for Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g (adjusted mean FEV_1 = 91.3%; mean FVC = 87.4) compared to a nonexposed comparison group (adjusted mean FEV_1 = 93.5%; mean FVC = 91.7) (Roegner et al., 1991). The 2,3,7,8-TCDD-related declines were small and were interpreted by the authors to be "subtle" and "not clinically significant." As expected, smoking appeared to have the greater influence on lung function.

7.13.9.1. *Comment*

In conclusion, case reports indicate that intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation. However, the findings from controlled epidemiologic studies do not support an association between 2,3,7,8-TCDD exposure and chronic effects on the respiratory system.

7.13.10. Renal Effects

There is little evidence in the animal or human data to suggest that exposure to 2,3,7,8-TCDD is related to renal or bladder dysfunction. In a single case report, a child exposed to 2,3,7,8-TCDD after contact with soil sprayed with contaminated waste oil was diagnosed with focal pyelonephritis (Kimbrough et al., 1977). After diagnosis and treatment,

the condition resolved with no reported recurrence. No major renal or bladder dysfunctions were noted among Air Force Ranch Hands (Lathrop et al., 1984, 1987; Roegner et al., 1991) or among TCP production workers from West Virginia (Suskind and Hertzberg, 1984) or New Jersey (Poland et al., 1971).

7.13.11. Reproductive Effects

Animal studies of reproductive effects have focused primarily on maternal 2,3,7,8-TCDD exposure and teratogenesis (Courtney and Moore, 1971; Courtney, 1976; Giavinni et al., 1983; Khera and Ruddick, 1973; Neubert and Dillman, 1972). Some ambiguity is introduced into the interpretation of results because of differences in study design, dosages, and species sensitivity. However, prenatal exposure to 2,3,7,8-TCDD has been associated with cleft palate and kidney abnormalities in rats (Khera and Ruddick, 1973) and mice (Courtney and Moore, 1971; Neubert and Dillman, 1972). Fetal resorption (Courtney, 1976) and reduced fertility (Giavinni et al., 1983) have also been observed in studies of maternal exposure.

Fewer studies have focused on the effects of dioxin on the male reproductive system or on the results of matings in which only the males were exposed to dioxin. Studies of male exposures have not provided evidence of changes in sperm characteristics or effects on the offspring (Lamb et al., 1980; Murray et al., 1979). Chapter 5 contains a more detailed account of the animal literature.

Thus, experimental research has emphasized maternally mediated reproductive effects of 2,3,7,8-TCDD while in humans, studies of paternal exposures have predominated. Moreover, assessment of exposed male animals has most commonly examined the effects of 2,3,7,8-TCDD on spermatogenesis, fertility, and sex organ development (Theobold and Peterson, in press), whereas studies of human males have mainly targeted effects on congenital malformations and recognized spontaneous abortions. Experimental research designed to corroborate human investigations may provide critically needed data to plug the gaps in our understanding of the mechanisms through which 2,3,7,8-TCDD exposure may operate to produce adverse reproductive events in humans.

The origin of concerns regarding a potential link between exposure to chlorinated dioxins and adverse reproductive events can be traced to early animal studies reporting increased incidence of developmental abnormalities in rats and mice exposed early in gestation to 2,4,5-T (Courtney and Moore, 1971). This was of grave concern, as the U.S. military's most widely used herbicide during the Vietnam conflict at that time, Agent Orange, was composed of approximately equal proportions by weight of the n-butyl esters of 2,4-D and 2,4,5-T. The latter is contaminated by 2,3,7,8-TCDD during manufacture.

One dilemma encountered when attempting to review the epidemiologic literature dealing with dioxins and reproductive effects is the categorization of studies of sufficient similarity to allow for comparative analysis. These studies vary greatly in the nature (occupational, environmental) and route (inhalation, digestion, absorption) of exposure; in the reproductive outcomes examined (often multiple end points were considered, and case definition differed across studies); in the assessment of parental exposure (maternal, paternal, or both); and in the timing of exposure relative to the pregnancy.

The examination of reproductive and developmental disorders poses several challenges to the researcher as compared with other health outcomes. First, to understand both normal and pathologic reproduction, evaluation should include paternal and maternal, and sometimes fetal, contributions. The recent increased interest in male-mediated reproductive toxicity emphasizes the need to consider the couple as the unit of analysis in many reproductive study settings.

The second challenge to researchers is the interrelatedness of the spectrum of reproductive end points available for study. Fecundity (the capability to conceive), fertility (the capability to produce live children), and early pregnancy loss (those conceptions that do not survive to be recognized by usual diagnostic methods) as related to 2,3,7,8-TCDD exposure have not been evaluated. Studies investigating early fetal loss and reduced fertility among couples where exposure to 2,3,7,8-TCDD is an issue are not available. Clearly, these end points affect the rates of reproductive outcomes occurring later in the reproductive spectrum.

Another feature of reproductive effects is the changing vulnerability of the developing organism throughout gestation. Exposure to a single teratogen throughout pregnancy may

result in different effects at various stages of gestation. The window of susceptibility varies among different teratogens; therefore, knowledge of the timing of exposure is critical in these studies.

Finally, although not restricted to studies of reproductive events, care must be given to the collection and analysis of confounding variables. These factors may need to be obtained for both mother and father, with attention to the timing of specific characteristics, such as changes in smoking or occupation during pregnancy. These are some points to keep in mind as the reader reviews the studies of 2,3,7,8-TCDD and reproductive effects presented here.

The reproductive effects of dioxins in humans were succinctly and elegantly reviewed by Hatch in 1984 (Hatch, 1984b). In her review, Hatch employed type of exposure, i.e., populations that were exposed occupationally, environmentally, or through industrial accident or military service, as the classification scheme for the research presented. The same approach has been followed in this review. The earlier investigations of 2,3,7,8-TCDD and reproductive effects, i.e., those conducted prior to 1984, are presented separately from the more recent studies. The development of assays in the mid-1980s to quantitate 2,3,7,8-TCDD in serum and adipose tissue, allowing individual measurements of exposure, warrants this dichotomy of the research.

7.13.12. Review of the Literature Prior to 1984

7.13.12.1. *Occupational Studies*

Epidemiologic studies of occupational dioxin exposure and reproductive effects have focused on potential paternally mediated effects, with exposures occurring at varying intervals relative to conception. Townsend et al. (1982) interviewed 370 wives of employees exposed to dioxins at the Dow Michigan Division in Midland, Michigan (63% of those eligible for the study), and 345 control wives of Dow employees who were not exposed to dioxin (62% of the eligible control pool). Exposure classification was determined by an industrial hygienist familiar with the processes performed at the plant. Employees were considered exposed to dioxin if they had been assigned for at least 1 month to specific jobs

associated with chlorophenol processes between 1939 and 1975. All outcomes were reported by the employee's spouse.

There was no systematic attempt to ascertain the reason(s) for the high refusal rate in both cohorts. "Unsolicited reasons" for refusal included divorce, death of spouse, or no pregnancies; no breakdown by cohort was provided. The possibility of differential rates of infertility or early pregnancy loss, as reflected by the reported absence of pregnancy, was not addressed in this study.

For the multiple end points of spontaneous abortion, stillbirth, birth defects, infant mortality, and childhood morbidity, no significant association between dioxin exposure and any adverse event was identified (Table 7-43). Given the very long interval when both exposure and event could have occurred and the minimum requirement for paternal exposure being 1 month at any time during the interval, an effect of exposure, if it existed, would have been diluted by this approach unless the damage was "irreparable," as it was defined in this report. The authors do not discuss whether time since last exposure was considered in the analysis.

In another study of occupational exposure to 2,4,5-T, Smith et al. (1982) evaluated 548 professional chemical sprayers and their spouses in New Zealand, ascertaining spraying activities from the males and reproductive histories from their spouses through a mailed questionnaire. A group of 441 agricultural contractors and their spouses served as controls. This study had impressive response rates of 89% for the exposed and 83% for the nonexposed groups. The investigators noted that wives of the chemical sprayers anecdotally reported assisting their husbands in spraying activities, some performing this task during their pregnancy. No associations between herbicide exposure and the outcomes of spontaneous abortion (OR=0.89, 95% CI=0.6-1.3) or congenital malformations (OR=1.2, 95% CI=0.6-2.4) were identified.

The questionnaires were completed in 1980 and elicited information on spraying activities and reproductive events that occurred between 1969 and 1980, a rather long period of time for recall of these events. Reported pregnancy outcomes were categorized into three groups based on whether the fathers had sprayed any chemicals at any time during, or prior to, the calendar year in which the pregnancy occurred and whether 2,4,5-T had been used.

Table 7-43. Results of Studies Examining the Effect of Dioxin on Reproductive Outcomes in Humans, 1984-1992

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Smith et al., 1982	548 male pesticide applicators who sprayed 2,4,5-T and other pesticides	441 agricultural contractors	Spraying of 2.4.5-T	Mailed survey/mailed survey	Total births	1,172	1,122		
					Congenital defect	13	9	1.19[a]	0.58-2.45
					Miscarriage[b]	43	40	0.89	0.61-1.30
					Stillbirth	3	0	---	---
Townsend et al., 1982	370 male chemical workers exposed to 2,4,5-T only and their spouses	345 employees not exposed to 2,4,5-T and their spouses	Working with chlorophenol processes	Interviewer-administered questionnaire/ interviewer-administered questionnaire	# Conceptions	418	2,031	---[c]	
					All fetal deaths	50	246	1.02	0.71-1.47
					Stillbirth	7	33	0.97	0.38-2.36
					Spontaneous abortions	43	213	0.96	0.65-1.42
					Infant deaths	6	39	0.82	0.30-2.09
					Health defects	32	155	0.93	0.60-1.43
					Congenital malformation	21	87	1.08	0.63-1.83

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Townsend et al., 1982 (cont.)	Male chemical workers exposed to any dioxins				Total conceptuses	737	2,031		
					All fetal deaths	100	246	1.03[a]	0.78-1.37
					Stillbirth	15	33	1.06	0.54-2.09
					Spontaneous abortions	85	213	1.03	0.77-1.39
					Infant deaths	9	39	0.63	0.27-1.39
					Health defects	52	155	0.85	0.60-1.21
					Congenital malformation	30	87	0.85	0.53-1.35
Mastroiacovo et al., 1988	2,900 births in Zones A, B, and R, Seveso, Italy, 1977-1981	12,391 births in study area outside Zones A, B, and R	TCDD cloud released from chemical plant accident	TCDD soil analysis/ Seveso Congenital Malformations Registry	Total birth defects	137	605	0.97	0.83-1.13
					Multiple birth defects	10	38	1.12	0.63-2.02
					Syndromes	5	29	0.74	0.33-1.63
					Major birth defects	67	343	0.83	0.67-1.04
					Minor birth defects	70	262	1.14	0.92-1.42
Stehr et al., 1986	68 persons residing in areas of high TCDD concentration	36 persons with no known contact with contaminated soil	Contact with TCDD-contaminated soil	EPA soil analyses for TCDD/interview	Infertility (males)	--	--	--	NS
					Impotence	--	--	--	NS
					Infertility (females)	--	--	--	NS
Hoffman and Stehr-Green, 1989	154 residents of Quail Run Mobile Home Park	155 residents nonexposed mobile park home	Contact with soil sprayed with TCDD for dust control	EPA soil analyses for TCDD/interview	Fetal deaths	--	--	--	NS
					Spontaneous abortions	--	--	--	NS
					Congenital malformations	--	--	--	NS

Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Stockbauer et al., 1988	402 pregnancies to exposed mothers	804 pregnancies to unexposed mothers (matched on maternal age and race, hospital and year of birth, plurality)	Contact with soil sprayed with TCDD for dust control	EPA soil analyses for TCDD/vital statistics and hospital records	Birth defects (all)	17	42	0.78	0.40-1.47
					Major birth defects	15	35	0.84	0.40-1.66
					Multiple birth defects	2	11	0.34	0.03-1.65
					Fetal deaths	4	5	1.60	0.32-7.43
					Infant deaths	5	5	2.00	0.46-8.69
					Perinatal deaths	6	9	1.33	0.39-4.20
					Low birth weight	27	36	1.59	0.89-2.81
					Very low birth weight	1	4	0.50	0.01-5.05
					IUGR	14	26	1.09	0.50-2.28
Erickson et al., 1984	4,929 infants from the Metropolitan Atlanta Congenital Defects Program	3,029 infants from Georgia Vital Statistics Records	Vietnam military service	Self-reported, and Exposure Opportunity Index/Birth defects registry and vital statistics	Total birth defects (96 subcategories also examined)	428	4,387	0.97	0.83-1.14
CDC, 1988d, 1989	7,924 Vietnam veterans	7,364 non-Vietnam veterans	Vietnam military service	Military records/self-reports	Total birth defects	826	590	1.3	1.2-1.4
					Spontaneous abortion	1,566	1,190	1.3	1.2-1.4
					Stillbirth	126	131	0.9	0.7-1.1
					Low birth weight	100	87	1.1	0.8-1.4
					Childhood cancer	25	17	1.5	0.8-2.8
					Sperm abnormalities:				
					concentration	51	20	2.3	1.2-4.3
					motility	91	58	1.2	0.8-1.8
					morphology	51	29	1.6	0.9-2.8
Substudy #1, CDC 1988d, 1989	1,791 offspring of Vietnam veterans	1,575 offspring of non-Vietnam veterans	Vietnam military service	Military records/self-report and hospital records verification	Total birth defects	130	112	1.0	0.8-1.4
					Low birth weight	51	37	1.1	0.7-1.8
					Perinatal mortality	58	54	1.0	0.7-1.5
					Suspected birth defects	21	21	0.9	0.5-1.7

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Substudy #2, CDC 1988d, 1989	127 offspring of Vietnam veterans	94 offspring of non-Vietnam veterans	Vietnam military service	Military records/self-report and hospital record verification	Cerebrospinal malformations	26	12	1.8	0.8-4.0
Stellman et al., 1988	2,858 Vietnam veterans	3,933 non-Vietnam veterans	Vietnam military service	Survey/ survey	Difficulty conceiving	349	363	1.2	NS
					Time to conception	4.4 years	4.4 years	--	NS
					Birth weight	--	--	--	NS
					Spontaneous abortion	231	195	1.3	1.4-2.0
Aschengrau and Monson, 1989	201 spontaneous abortion cases at Boston Hospital for Women	1,119 full-term births at Boston Hospital for Women	Vietnam military service	Military records/ hospital records	Spontaneous abortion	8	44	0.9	0.42-1.9
Aschengrau and Monson, 1990	966 infants with late adverse pregnancy outcomes at Boston Hospital for Women	998 normal term infants at Boston Hospital for Women	Vietnam military service	Military records/ Hospital records	Total birth defects	55	656	1.3	0.9-1.9
					≥1 Major malformation	18	151	1.8	1.0-3.1
					Minor malformation	11	189	0.9	0.5-1.7
					Stillbirths	5	51	1.5	0.4-3.9
					Neonatal deaths	3	36	1.2	0.2-4.2
Wolfe et al., 1992b	2,533 conceptions among 791 Ranch Hand personnel	2,074 conceptions among 768 non-Ranch Hand personnel	Spraying/ handling of Agent Orange	Serum TCDD levels/ hospital and medical records	Total birth defects				
					≤10[k]	202.1[l]	208.0	0.96[m,a]	0.69-1.34
					15-≤33.3	293.1		1.58	1.10-2.27
					>33.3	193.8		0.92	0.64-1.32

Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Wolfe et al.. 1992b (cont.)					Nervous system anomalies				
					≤10[k]	0.0[l]	3.1	---	
					15-≤33.3	5.7		1.88[m,o]	0.20-18.3
					>33.3	13.2		4.37[m,o]	0.87, 21.8
					Respiratory system anomalies				
					≤10[k]	7.1[l]	2.0	3.5[m,o]	0.49-25.0
					15-≤33.3	5.7		2.83	0.26-31.4
					>33.3	4.4		2.17	0.20-24.0
					Digestive system anomalies				
					≤10[k]	21.3[l]	24.5[l]	0.83	0.31-2.23
					15-≤33.3	34.5		1.30	0.48-3.51
					>33.3	17.6		0.64	0.21-1.91
					Genital anomalies				
					≤10[k]	3.5[l]	18.3[l]	0.19	0.03-1.43
					15-≤33.3	51.7		2.92	1.29-6.61
					>33.3	13.2		0.72	0.21-2.46
					Urinary system anomalies				
					≤10[k]	14.2[l]	12.2[l]	1.16	0.37-3.63
					15-≤33.3	34.5		2.88	1.07-7.79
					>33.3	22.0		1.82	0.63-5.22
					Musculoskeletal deformities				
					≤10[k]	120.6[l]	134.6[l]	0.88[m,n]	0.59, 132
					15-≤33.3	143.7		1.08	0.68-1.71
					>33.3	105.7		0.76	0.48-1.21

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Wolfe et al., 1992b (cont.)					Anomalies of the skin				
					≤10[k]	17.7[l]	21.4[l]	0.76	0.25-2.26
					15-≤33.3	34.5		1.83	0.72-4.70
					>33.3	8.8		0.46	0.11-1.95
					Circulatory system and heart anomalies				
					≤10[k]	14.2[l]	16.3	0.85	0.27-2.69
					15-≤33.3	46.0		2.16	0.81-5.74
					>33.3	8.8		0.32	0.04-2.52

[a]Relative risk.
[b]Rate: 86/1,000 births in applicators; 93/1,000 births in agricultural contractors (controls).
[c]Adjusted for mother's age at time of birth, birth control methods, labor and delivery complications, medical conditions and medications during pregnancy, smoking and alcohol use during pregnancy, high job risk, and gravidity.
[d]Rate: Zone A: 0; Zone B: 57.5/1,000 births; Zone R: 45.1/1,000 births; zone non-ABR: 48.8/1,000 births.
[e]90% CI.
[f]Zones A and B.
[g]Zones A and B vs. non-ABR.
[h]Controls matched on maternal age, race, hospital of birth, plurality, and year of birth.
[i]Odds ratio adjusted for veteran's age at birth, year of entry in army, enlistment status, general technical test score, military occupational specialty, years between entry and birth, maternal age, and gravidity.
[j]Children born after the father was stationed in Southeast Asia (SEA).
[k]Logit (p) = $\beta_0 + \beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3$, where p = probability of an adverse reproductive outcome; d_1, d_2, d_3 are indicators for the dioxin categories: Unknown (Ranch Hands with up to 10 pg/g current dioxin), Low (Ranch Hands with more than 15 pg/g of lipid and up to 33.3 pg/g of lipid current dioxin), and High (Ranch Hands with more than 33.3 pg/g of lipid current dioxin).
[l]Rate of abnormals.
[m]Unadjusted.
[n]Adjusted analysis not statistically significant.
[o]No adjusted analysis: total defects < 10.

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There were 1,122 births reported among the 441 control spouses and 1,172 among the 548 spouses of the exposed sprayers. If all other factors were considered equivalent in both cohorts (maternal age, socioeconomic status, and maternal smoking histories were shown to be similar), then there appear to be 220 fewer births observed in the exposed group than might be expected.

This study is limited by the lack of information on the total number of conceptuses and the high probability of exposure to chemicals other than 2,4,5-T. While exposure levels were not quantitated in this study, a later study of a subset from this same population was conducted to estimate 2,3,7,8-TCDD exposure in this group.

In 1988, nine pesticide applicators with the greatest number of years and months per year of pesticide application were selected for serum 2,3,7,8-TCDD analysis (Table 7-44). The mean serum 2,3,7,8-TCDD level, adjusted for total lipids, among the cases (53.3 pg/g) was 10 times that of age-matched controls (5.6 pg/g). However, exposure in the reproductive study was based on self-reports of pesticide application, and research has demonstrated repeatedly that self-reports do not correlate with documented serum 2,3,7,8-TCDD levels (Needham et al., 1991). Therefore, it would be helpful if serum levels could be obtained from a subset of those applicators with lower self-reported exposures.

A clinical epidemiology study conducted in 1979 examined workers involved with the manufacture of 2,4,5-T between 1948 and 1969 in Nitro, West Virginia (Suskind and Hertzberg, 1984). All active and retired plant employees exposed to the 2,4,5-T process during that 22-year interval comprised the eligible pool of "exposed" subjects. The control group consisted of current and former plant employees who were never associated with the 2,4,5-T process, according to company records. The response rates for these cohorts were 61% (N=204) and 46% (N=163), respectively.

A reproductive history was obtained from these male employees during an interview and clinical examination. No attempt was made to verify reports of live births, infant deaths, miscarriages, birth defects, and stillbirths with the spouses or through medical records. There were no significant differences in rates of any adverse outcome by exposure status. Given the poor response rates, crude measure of exposure, and lack of verification of

Table 7-44. 2,3,7,8-TCDD Levels (pg/g of Lipid) for Selected Populations

Author	Study population	Specimen	Range	Mean	Median
Mocarelli et al., 1991	Seveso, Italy residents: 10 Zone A 10 former Zone A 10 non-ABR Zone	Serum	 828 - 56,000 1770 - 10,400 nd-137	 19,144 5,240 -	 14,000 4,540 -
Patterson et al., 1986b	39 Missouri residents with history of TCDD exposure	Adipose tissue	2.8 - 750	79.7	17.0
	57 Missouri residents with no known TCDD exposure		1.4 - 20.2	7.4	6.4
Smith et al., 1992	9 New Zealand pesticide applicators	Serum	3.0 - 131.0	53.3	37.6
	9 controls		2.4 - 11.3	5.6	9.3
CDC, 1988	646 Vietnam ground combat troops with service in heavily sprayed areas	Serum	nd - 45	4.2	3.8
	97 non-Vietnam veterans		nd - 15	4.1	3.8
Kahn, 1988	10 "heavily exposed" Vietnam veterans	blood (per lipids) adipose tissue	- -	46.3 41.7	25.1 15.4
	10 Vietnam veterans with "little or no" exposure	blood (per lipids) adipose tissue	- -	6.6 5.1	5.3 5.4
	7 non-Vietnam veterans	blood (per lipids) adipose tissue	- -	4.3 3.2	3.9 3.5

Table 7-44. (continued)

Author	Study population	Specimen	Range	Mean	Median
Schecter et al., 1989	26 Vietnam veterans	adipose tissue	nd - 11	5.8	-
Kang, 1991	36 Vietnam veterans	adipose tissue	-	13.4	10.0
	79 Non-Vietnam veterans		-	12.5	11.4
	80 Civilians		-	15.8	11.8
Roegner et al., 1991	872 Ranch Hands	serum	0-617.8	-	12.8
	1060 Controls		0-54.8	-	4.2
Phuong, 1989b	Vietnamese Populations:	adipose tissue	nd - 103	23	11.3
	9 OB/GYN patients from a South Vietnam hospital				

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paternally reported reproductive histories, this study was not likely to detect an association between 2,3,7,8-TCDD and reproductive events if one existed.

7.13.12.2. *Environmental Studies*

The problem of documentation of exposure is perhaps of even greater concern in studies of subjects environmentally exposed to dioxins that are lacking individual 2,3,7,8-TCDD measures. The route of exposure (inhalation, ingestion, absorption), length and intensity of exposure, and the timing of the exposure are more difficult to estimate in free-living populations compared with workers in an occupational setting.

Selection bias is also a critical concern in environmental studies because there is no equivalent to company records in the occupational setting that defines the population at risk. Proximity of residence to a contaminated site was generally the best option available for identifying the population at risk of exposure. Issues such as length of time at that residence, the amount of time the subject spent in the home, and the occurrence of the contamination episode relative to the time spent at home may have greatly affected the degree of exposure.

Volunteer bias, which is inherent in studies that rely on subjects responding to publicized requests for participation, is an additional concern in studies of this nature. Moreover, it is extremely difficult to conduct epidemiologic investigations under crisis situations such as the industrial accident that occurred in Seveso, Italy. Given these limitations, the efforts of these investigators have provided valuable impetus to the refinement of study designs and the development of more sophisticated techniques to explore this issue.

7.13.12.3. *The Seveso, Italy, Dioxin Accident of 1976*

The 1976 accident at a chemical plant near Meda, Italy, in which effluent containing 2,3,7,8-TCDD among other contaminants was released, has been well described (Pocchiari, 1979; Reggiani, 1978). Following the reactor release, an extensive surveillance system was initiated to monitor the health of the exposed population. Rates of reproductive events were examined by "zones" of exposure, as described above.

The Seveso Congenital Malformations Registry enrolled all live births and stillbirths occurring January 1, 1977, through December 31, 1982, to women residents of Zones A, B, R, and non-ABR (Mastroiacovo et al., 1988). A total of 15,291 births (live and still) and 742 birth defects were recorded: Zone A, 26 births and no birth defects; Zone B, 435 births and 25 birth defects (57.5/1,000 births); Zone R, 2,439 births and 110 birth defects (45.1/1,000 births); Zone non-ABR, 12,391 births and 605 birth defects (48.8/1,000 births). Birth defects were confirmed by medical records. Relative risk estimate for total defects comparing Zones A+B with Zone non-ABR is 1.2 (90% CI=0.88-1.64) (Table 7-43). The rate of birth defects occurring early in the observation period (first quarter of 1977) did not differ from the rate of birth defects occurring later in the observation period (data not shown), and there were no discernible patterns of defects within or between the exposure groups.

To document an increase in the rate of an event, sound baseline information is required. No reliable information regarding rates of birth defects in this area was available until the establishment of the Seveso Congenital Malformations Registry 6 months after the exposure incident. Thus the potential contribution of 2,3,7,8-TCDD to the congenital malformation rate cannot be separated from improved case ascertainment.

The influence of both spontaneous and induced abortions on the birth defect rate is likewise unknown. As with congenital malformations, reliable background data for spontaneous abortion in the study area were not available. The impact of induced abortions sought after the accident is difficult to estimate, particularly since abortion was illegal in Italy in 1976. The birth rate for the entire study area declined between 1976 and 1980, and the small number of conceptions available for study limits the power of these studies to detect an association between 2,3,7,8-TCDD exposure and spontaneous abortion and birth defects.

Another adverse outcome that has not been described in most epidemiologic studies is the effect of 2,3,7,8-TCDD on the chromosome. Pocchiari et al. described one study in which 30 therapeutic and four spontaneous abortions from the Seveso area were examined (Pocchiari, 1979). There were no indications of mutagenic, teratogenic, or embryotoxic effects that could be attributed to 2,3,7,8-TCDD exposure. However, it was difficult to determine maternal exposure status for any of the cases. A more recent study examined the

association between 2,3,7,8-TCDD and cytogenetic abnormalities in fetuses from abortions induced shortly after the Seveso accident (Tenchini et al., 1983). The frequency of aberrant cells and the mean number of aberrations per damaged cell were significantly higher in exposed versus unexposed fetuses. However, the paper omits important data, including whether the fetus was conceived before or after 2,3,7,8-TCDD exposure, how long after exposure the abortion occurred, the zone in which the mother and father resided at the time of conception and pregnancy, and tissue 2,3,7,8-TCDD concentration in the fetuses. No other studies have examined fetal tissue in this manner to corroborate these data. This approach offers exciting possibilities for future research, however, as genetic techniques in the area of DNA-adduct analyses become more widely available.

7.13.12.4. *Studies of Exposure to Agent Orange by Military Veterans and Vietnamese Civilians*

The problem of exposure documentation has also been a highly controversial issue in studies of potential exposure to Agent Orange in Vietnam and adverse health effects among Vietnam veterans and residents of Vietnam and their offspring. An early study among the Vietnamese population encompassed a 10-year period in Vietnam from 1960 to 1969 (Cutting et al., 1970). Exposure was dichotomized into pre- or light-spraying years from 1960 to 1965 and heavy-spraying years from 1966 to 1969. A total of 480,087 births, 16,166 stillbirths, 2,866 hydatidiform moles, and 2,355 congenital malformations of all types were examined in this study. Pregnancy outcome data were collected from 22 hospitals.

Increases in the rates of stillbirths, molar pregnancies, and congenital malformations were noted in the coastal plain and delta areas following heavy spraying, although the authors emphasized the slight downward trend observed for all outcomes in the countrywide rates. Several biases in this sampling approach severely limit the interpretation of the study's findings. The births examined were not representative of the births in the country during that period. In addition, the hospital records were incomplete, and transport of the mothers to the selected hospitals resulted in uncertainty regarding maternal residence during the pregnancy.

A second investigation conducted in Vietnam used the HERBS tape (military records detailing Agent Orange spraying missions) covering the period from 1965 to 1971 to determine maternal exposure status according to area of residence (Kunstadter, 1982). HERBS data were matched to hospital records indicating the date of birth to imply "estimated date of conception" and to maternal residence at birth to imply "potential for maternal exposure." Birth outcome data were collected from hospital records, which as in the above study were subject to inaccuracies and incompleteness.

No association between spraying of Agent Orange and any type of birth defect or perinatal mortality was noted. Although cleft lip defects increased in proportion to other malformations during the heavy spraying period, the total number of birth defects declined and continued to decrease after spraying activities ceased.

Finally, Australian investigators examined the relationship between service in Vietnam during 1962-1972 and birth defects (Report to the Minister for Veterans' Affairs, 1983). Cases were infants born with any of a defined set of congenital malformations in any of 34 hospitals in New South Wales, Victoria, and the Australian Capital Territory from 1966 to 1979. Control infants were matched to cases on maternal age, hospital, and time of birth, yielding 8,500 matched pairs for analysis.

Fathers of case and control infants were matched against a list of members who had served in the Australian Army during the specified time period. No associations were detected for Vietnam service and total birth defects (OR=1.02, 95% CI=0.8-1.3) or for any of the approximately 100 birth defects examined. Length of service in Vietnam, time between deplanement and conception, and Vietnam service prior to and following conception were considered in the analysis.

A critical point that should be emphasized regarding the Australian study is the assessment of service in Vietnam as the exposure of interest. The author clearly stated that investigations had indicated that exposure to herbicides was "infrequent and probably very low in Australian troops in Vietnam; the study does not exclude possible effects of herbicides in situations of substantial exposure" (Report to the Ministry for Veterans' Affairs, 1983).

A series of unpublished studies conducted by Vietnamese investigators was reviewed by Hatch (1984a) and should be mentioned in this review. While these reports also have

limitations, including incomplete background data for rates of reproductive events and sparsity of epidemiologic details provided in the studies, the assessment of Vietnamese populations offered the opportunity to evaluate pregnancies with various patterns of parental exposure.

Investigations conducted in northern Vietnam assessed pregnancies with no maternal exposure to spraying activities; paternal exposure was presumed to have occurred only when the father had performed military service in the south.

Studies of couples in southern Vietnam compared reproductive outcomes observed in sprayed versus nonsprayed areas and represent potential associations between either maternal and/or paternal herbicide exposure and risk of adverse pregnancy outcome.

Three studies examined presumed paternal herbicide exposure and birth defects among pregnancies in northern Vietnam. Lang and Van compared the frequency of birth defects during the period 1975-1978 as a function of the father having served in south Vietnam (Hatch, 1984a). Among 2,547 offspring whose fathers had never served in the south, the birth defect rate was 6 per 1,000 (N=15). Among 511 offspring whose fathers had served in the south, the rate was 29 per 1,000 (N=15). Similar results were obtained in a study by Lang et al. at unspecified agricultural and handicraft cooperatives in northern Vietnam (Hatch, 1984a). The congenital malformation rate among "exposed" pregnancies (paternal service in southern Vietnam) was 23-26 per 1,000 (71 or 82 of 3,147). In comparison, the rate among the unexposed pregnancies was 5 per 1,000 (10 of 2,172).

In what is perhaps the most stringent of the Vietnamese studies by Can et al., 40,064 women from three rice-growing districts in northern Vietnam were assessed (although few details are provided on the method of selection) (Hatch, 1984a). All of the women were married and pregnant at least once during the war and had no history of tuberculosis, syphilis, or malaria or of using antibiotics or hormones during pregnancy. "Detailed" interviews were conducted by physicians and midwives, and district health records were consulted in an attempt to validate reported pregnancies and outcomes. Only pregnancies conceived during the conflict were considered in this study.

There were a total of 121,993 pregnancies among the 29,041 women whose spouses were "nonexposed" and 32,069 pregnancies among the 11,023 women whose spouses had

served in the south. Service in south Vietnam was associated with an increased rate of spontaneous abortion ($p=0.05$). The increased rate of congenital malformations among the exposed pregnancies (0.6%) compared with the unexposed (0.4%) was marginally significant ($p=0.10$).

In attempt to confirm this finding, these investigators conducted a case/control study in which they examined a random sample of 61 families of children who had survived with a birth defect. A control group of 183 families of normal children matched on maternal age, number of deliveries, living environment, and age was selected. Forty-nine percent of children with a birth defect had a father who had served in south Vietnam compared with 21% of the children without malformations, yielding an odds ratio of 3.6.

Additional unpublished studies conducted in Vietnam by Vietnamese investigators were reviewed by Constable and Hatch (1985). The reader is referred to this source for details of the studies. It was concluded that studies of presumptive paternal "herbicide" exposure prior to or at conception were suggestive of a relationship with congenital malformations. The evidence for an association with spontaneous abortion was "less convincing," and for molar pregnancies no relationship appeared to exist. Those studies that examined both maternal and paternal "herbicide" exposure were also suggestive of a relation to birth defects as well as spontaneous abortion, stillbirths, and molar pregnancies. Follow-up studies by these Vietnamese investigators supported these earlier findings (Huong et al., 1989; Phuong et al., 1989b).

In the next section, the impact of assays to measure individual levels of 2,3,7,8-TCDD is discussed, and studies of 2,3,7,8-TCDD exposure and reproductive effects that have been published since 1984 are presented.

7.13.13. Review of the Literature From 1984 to 1992

During the interval since 1984, assays that had been developed to measure TCDD in serum and adipose tissue were being tested and refined. Several investigators have since used these assays in (usually small) subsets of their study populations to describe exposure to 2,3,7,8-TCDD in their total sample and also in attempts to validate their assumptions regarding magnitude of exposure for their study subjects. Subsets were generally selected to

represent subjects designated as "high" versus "low" exposure by the study investigators, utilizing various information sources including self-reports, company or military records, etc. Table 7-44 presents the results of the exposure analyses (Mocarelli et al., 1991; Patterson et al., 1986b; Smith et al., 1992; Centers for Disease Control Veterans Health Studies, 1988; Fingerhut et al., 1991a; Schecter et al., 1989; Kahn et al., 1988; Kang et al., 1991; Roegner et al., 1991; Phuong et al., 1989b).

These data illustrate wide variability in groups presumed to have been exposed to 2,3,7,8-TCDD at levels above background ($\leq$20 pg/g). For example, the mean and median serum levels of Vietnam ground combat troops with service in areas heavily sprayed with Agent Orange did not exceed the levels found in the U.S. general population. There is evidence for significantly higher exposure to 2,3,7,8-TCDD among certain subgroups of Vietnam veterans (Kahn et al., 1988; Roegner et al., 1991) as well as residents of Vietnam (Phuong et al., 1989b), Seveso (Mocarelli et al., 1991), and Missouri (Patterson et al., 1986b), and occupational groups (Fingerhut et al., 1991a; Beck et al., 1989).

It is also important to note that with the exception of the Ranch Hand study, these subsets were selected to describe 2,3,7,8-TCDD exposure in the total study sample and not for an examination of the relationship between 2,3,7,8-TCDD and reproductive events. In addition, the data from the Ranch Hand population indicated a serum 2,3,7,8-TCDD half-life of 7.1 years (Pirkle et al., 1989), but serum samples in this group were collected and analyzed at 11- and 15-year intervals following exposure. Questions regarding the impact of initial dose, age, gender, and pregnancy itself on half-life in humans remain unanswered at this time.

7.13.14. Environmental Studies

7.13.14.1. *The Times Beach, Missouri, 2,3,7,8-TCDD Episode*

In 1971, a waste oil dealer in Missouri disposed of waste sludge containing approximately 29 kg 2,3,7,8-TCDD by mixing it with waste oils as a dust control spray, which was subsequently distributed throughout the state. General media announcements from health officials were made, urging persons potentially exposed to 2,3,7,8-TCDD to participate in a survey and health screening process. People were warned of their potential

exposure by virtue of their residence, employment, or engagement in recreational activities in the contaminated sites. A pilot study was initiated in 1983, in which a subset of those persons who had responded to the media announcements was assessed (Stehr et al., 1986). Approximately 800 completed questionnaires were screened to select participants; it was not clear if this number represents all of the questionnaires that had been returned up to that time.

This phase of the study was intended to identify potential problems for future investigations. Persons determined to be at "high" versus "low" risk for dioxin exposure, based on their completed surveys, were selected. "High risk" was defined as either (1) reported residence or occupation in areas with TCDD levels between 20 and 100 ppb for at least 2 years or in areas with TCDD levels >100 ppb for at least 6 months, or (2) participation in activities requiring close contact with soil in areas with TCDD concentrations as described for similar periods of time. "Low-risk" persons were determined to have had no access to or "regular high-soil-contact activities" in any known contaminated area. Controls were frequency matched with the high exposed group on type of exposure site, age, sex, race, and socioeconomic status.

Sixty-eight "high-risk" persons (83% of those eligible) and 36 "low-risk" persons (90% response) were evaluated through physical, neurological, and dermatological examinations, laboratory tests, and interviews.

Information on reproductive outcomes was obtained during the interview administered to the subjects "or their nearest relative." None of the outcomes observed differed significantly by exposure status (Table 7-43), although high-risk women had a later mean age at menarche ($p=0.06$). A total of 30 births were available for assessment in this sample.

The following year, a more intensive study was undertaken to test the results found in the pilot study (Hoffman et al., 1986). The exposed group consisted of residents of the Quail Run Mobile Home Park in Gray Summit, Missouri, where TCDD levels in the soil were measured at up to 2,200 ppb. Data on 95 of the approximately 207 households in the park were available; 154 persons (74%) who were both "eligible and interested" agreed to participate. The unexposed group consisted of 155 residents of a nonexposed trailer park, representing 77% of those "both eligible and interested" in participation.

The protocol was similar to that employed in the pilot study described above. The authors reported that "no differences were found between the exposed and unexposed groups in the frequency of reproductive disorders or adverse pregnancy outcomes, such as fetal deaths, spontaneous abortions, and children with congenital malformations." No sample sizes or statistical test results for any of the outcomes were reported. Clearly, these studies of Missouri residents were not designed to investigate the relationship between dioxin exposure and reproductive outcomes, and not very much can be learned about this association from these results.

In a retrospective cohort study by Stockbauer et al. (1988), the association between 2,3,7,8-TCDD and reproductive outcomes was examined among residents of contaminated areas in Missouri. All live births and stillbirths that occurred in the nine residential areas identified as contaminated with TCDD during the period 1972-1982 were identified through Missouri vital statistics records. A total of 402 births were examined from six residential areas. TCDD levels in the soil from these six areas ranged from 241 to 2,200 ppb. 2,3,7,8-TCDD levels were not reported for the three areas where no births occurred during this interval, which would have been of interest to note.

A reference group of 804 unexposed births, matched for maternal age and race, hospital and year of birth, and plurality, was selected from the vital statistics records. Medical records were abstracted to ascertain birth defects in the matched sets. In addition, the births were linked to a statewide birth defect register that had been recently initiated. Data on several potentially confounding variables were obtained from birth certificates.

The exposed mothers tended to be less educated, had more children, were more likely to be in the extremes of the prepregnant weight distribution, and were more likely to smoke cigarettes. Statistical testing for these differences was not reported. Moreover, statistical adjustment for these potential confounders was performed only in the birth weight analyses, although there was little change in the birth weight risk ratios after adjustment.

Increased risk ratios were reported in the exposed group for infant death (OR=2.0), fetal death (OR=1.6), and perinatal death (OR=1.3), low birth weight (AOR=1.5), and several subcategories of malformations, although none of these findings achieved statistical significance (Table 7-43).

Two approaches were used to determine a dose-response relationship. In the first, the study data set was matched against the Missouri central listing of dioxin-exposed persons, which yielded 98 of the exposed mothers and none of the control mothers. These 98 women were then dichotomized into "high" (N=20) or "low" (N=78) exposure groups. High exposure was defined as residence for at least 6 months in areas with $\geq$100 ppb TCDD in the soil or $\geq$2 years in areas with TCDD levels from 20 to 100 ppb. Low exposure was defined as residence in areas with similar TCDD levels as described above but for less than the required time period or residence at sites with 1-19 ppb TCDD. No evidence for a dose-response relationship was observed with this analysis.

The second approach categorized the births into two different intervals relative to the spraying of the soil with the TCDD-contaminated sludge in 1971 to 1973. When births in the 1972-1974 period were compared with births from 1975 to 1982, the authors reported that "the only birth outcomes with higher risk ratios in the earlier time period were very low birth weight, birth defects, and major birth defects." None of these observations were statistically significant, but sample sizes and testing results were not provided in the paper.

The authors acknowledged the possibility of exposure misclassification and also the "modest" power of the study to detect associations due to the small sample size.

7.13.14.2. *Studies of Vietnam Experience in Ground Troops and Ranch Hands Published 1984-1992*

7.13.14.2.1. *Evaluation of exposure*

Evidence from earlier studies to determine if Agent Orange exposure increased the risk of adverse pregnancy outcomes among Vietnam veterans has been described as "sparse, sometimes off the point, sometimes conflicting..." (Hatch and Stein, 1986). The general dissatisfaction with these studies had a common factor: the lack of a valid measure of dioxin exposure. Once the assays to document individual 2,3,7,8-TCDD exposure became available and served as the gold standard for assessing the exposure assumptions made by study investigators, this concern regarding exposure misclassification was shown to be justified. Until 1992, when the first study to examine reproductive outcomes among Vietnam veterans based on individual exposure measurements of 2,3,7,8-TCDD was published, this remained

the major criticism of the research. However, even though 2,3,7,8-TCDD serum levels were available for reanalysis of the 1984 data of the Ranch Hand study, the lack of a good estimate of 2,3,7,8-TCDD level at the time of conception continues to instill uncertainty about the findings.

7.13.14.2.2. ***Exposure indices***

7.13.14.2.2.1. ***The CDC case-control study's exposure opportunity index.*** In the early 1980s, the CDC conducted a large case-control study that examined the relationship between service in Vietnam and risk of congenital malformations (Erickson et al., 1984). Although service in Vietnam was the major exposure variable, two additional measures of exposure were assessed. Vietnam veterans were asked if they believed they had been exposed to Agent Orange. In addition, an exposure opportunity index (EOI), developed by the Army Agent Orange Task Force, assigned a score estimating the likelihood of exposure based on places and times of Vietnam service. These scores ranged from minimal (1) to high (5) opportunity for Agent Orange exposure.

The CDC then reported on a study, using the serum assay as the standard, to evaluate the validity of both self-reported exposure to Agent Orange and use of military records to fomulate the EOI used in the case-control study of military service in Vietnam and birth defects. The results revealed a poor correlation (no correlation coefficient was provided) between both of these exposure estimates and serum TCDD levels (Centers for Disease Control Veterans Health Study, 1988). In addition, the distributions of serum 2,3,7,8-TCDD levels were nearly identical (median = 3.8 ppt) among 646 ground combat troops who had served in heavily sprayed areas compared with 97 veterans who had never served in Vietnam.

In a separate study, Kahn et al. (1988) measured serum 2,3,7,8-TCDD levels in 10 Vietnam veterans who reported that they had handled Agent Orange "regularly" while in Vietnam, 10 Vietnam veterans with little or no exposure to Agent Orange, and 27 Vietnam-era veterans. The levels of serum 2,3,7,8-TCDD among those men who had handled Agent Orange were significantly elevated (median = 25.1 pg/g blood fat) compared with the

Vietnam control veterans (median = 5.3 pg/g) and the Vietnam-era veterans (median = 3.9 pg/g) ($p<0.01$).

7.13.14.2.2.2. ***The Baseline Ranch Hand study's exposure index.*** In 1984, the U.S. Air Force released preliminary results of a 20-year study designed to examine the personnel responsible for conducting the aerial spraying of herbicides in Vietnam (Lathrop et al., 1984). The analyses in this baseline study were based on cohort status, i.e., Ranch Hands ("exposed") versus controls ("nonexposed"). In an attempt to determine a link between exposure and clinical end points, an exposure index was developed to estimate individual 2,3,7,8-TCDD exposure. The exposure index developed for this study was defined as the product of a TCDD weighting factor and the gallons of TCDD-contaminated herbicides sprayed during the veteran's tour divided by the number of Ranch Hands sharing his duties during his tour.

In 1988, a report describing a U.S. Air Force and CDC collaborative pilot study utilizing the serum 2,3,7,8-TCDD assay among 200 Air Force Ranch Hand personnel was published. It was noted that the Ranch Hand personnel had significantly higher serum 2,3,7,8-TCDD levels than controls (Wolfe et al., 1988). These data also indicated that the Ranch Hands as a whole were not as highly exposed to 2,3,7,8-TCDD as compared with the NIOSH cohort (Piacitelli et al., 1992) and the Seveso population (Mocarelli et al., 1991).

In 1992, the report describing reproductive outcomes among Ranch Hand personnel became available (Wolfe et al., 1992b). In addition to outcome verification, serum 2,3,7,8-TCDD levels were measured in a sample of Ranch Hands (N=791) and the comparison population (N=942). A comparison of the exposure index used in the baseline Ranch Hand study with individual 2,3,7,8-TCDD levels revealed "considerable misclassification" among the study subjects.

As a result of these investigations, it became clear that the likelihood of exposure misclassification in studies of the relationship between 2,3,7,8-TCDD and reproductive events, without direct measures of individual exposure, casts considerable doubt as to the validity of the findings.

An equally important finding resulting from these investigations was the inability of the exposure indices to classify exposure status in individuals (Needham et al., 1991). 2,3,7,8-TCDD analyses in small subsets of the total study sample, selected on the basis of presumed exposure as defined by an "exposure index" (either military or government records, self-reports, or 2,3,7,8-TCDD levels in soil samples), have demonstrated that the exposure classification schemes employed in these studies were not valid. Therefore, studies that defined exposure as paternal military service in Vietnam should be evaluated without inference to 2,3,7,8-TCDD exposure.

7.13.15. Study Results

7.13.15.1. *Atlanta Congenital Defects Program Study*

In 1984, the CDC released the findings from the large case-control study that examined the relationship between service in Vietnam and risk of congenital malformations (Erickson et al., 1984). Case-group babies were infants with serious structural malformations born between 1968 and 1980 and registered with the Metropolitan Atlanta Congenital Defects Program (MACDP). Of 7,133 eligible cases, maternal interviews were obtained for 4,929 (69%). Control babies were selected from Georgia vital statistics records and were frequency matched to cases on race and year and hospital of birth. Of 4,246 eligible controls, maternal interviews were obtained for 3,029 (71%). Paternal interview rates were 56% and 57%, respectively.

While response rates were similar by case status overall, among nonwhites significantly more cases were not interviewed. The major reason for nonparticipation was the inability to locate subjects rather than subjects refusing to enroll.

Among the children with congenital malformations, 428 (9%) were fathered by Vietnam veterans and 4,387 (91%) were fathered by non-Vietnam veterans; identical percentages were noted among the control infants. The odds ratio for service in Vietnam and birth defects of any type was 0.97 (95% CI=0.83-1.14). Odds ratios were also calculated for 96 separate categories of birth defects, with no significant associations observed.

The EOI developed for this study (as described above) also was not associated with total birth defects. However, significant associations were observed for spina bifida, cleft lip

with or without cleft palate, neoplasms, and coloboma. With the exception of the last defect, all three also showed evidence of a dose-response relationship.

For the analysis that examined potential associations between self-reported Agent Orange exposure and birth defects, the frequency of exposure among fathers and each type of malformation was compared with the frequency among fathers and all other defects in an attempt to reduce recall bias. Twenty-five percent (N=74) of the Vietnam veterans reported that they believed they were exposed to Agent Orange during their service in Vietnam. The analysis of self-reported exposure and birth defects was negative on all counts, in contrast to the EOI analysis, which found significant associations as described above. However, the small numbers of cases of many individual defects resulted in a virtual lack of power to detect any associations for these specific defects.

7.13.15.2. *CDC Vietnam Experience Study*

As part of a separate, large, multifaceted study mandated by Congress, the CDC evaluated the risk of service in Vietnam and adverse reproductive outcomes (Centers for Disease Control Vietnam Experience Study, 1988d, 1989). The Vietnam Experience Study protocol involved two phases. In the first phase, a random sample of male veterans who met eligibility criteria related to military service was selected for a telephone interview.

Of the eligible "exposed" group, i.e., those veterans who had served in Vietnam, 84% (N=7,924) agreed to participate, and 84% (N=7,364) of the nonexposed (those who had not served in Vietnam) were enrolled. Of these 15,288 veterans who completed the telephone interview, a random sample was selected for the second phase, which consisted of a comprehensive medical examination. For this phase, the response rates were 75% (N=2,490) for the Vietnam veterans and 63% (N=1,972) for the non-Vietnam veterans group.

During the telephone interview, veterans were questioned about miscarriage, induced abortion, ectopic pregnancy, live births, stillbirths, birth defects, as well as leukemia and other childhood cancers, and major health problems or impairments during the first 5 years of life.

A preliminary analysis of the interview data revealed that the Vietnam veterans had reported 40-50% more birth defects than the non-Vietnam veterans. In addition, a difference between the cohorts for certain subcategories of malformations, including spina bifida, cleft lip with or without cleft palate, and hydrocephalus, was noted. Therefore, a substudy was conducted to compare the rates of total birth defects in Vietnam and non-Vietnam veterans as these events were recorded on hospital records.

The eligible sample for this substudy consisted of 2,282 veterans who had not yet received their medical examinations, as it would be easier to collect the additional information required and permission to obtain hospital records for their offspring from this group. Hospital records were obtained for 92% (N=1,791) of the offspring of Vietnam veterans and 91% (N=1,575) of the offspring of non-Vietnam veterans.

When birth defects were identified from hospital records, there was no association of Vietnam service with total, major, minor, or suspected birth defects. From the telephone interview data, the odds ratio for Vietnam service and total birth defects was 1.3 (95% CI=1.2-1.4); in the hospital records substudy, the odds ratio was 1.1 (95% CI=0.7-1.8). It was concluded that this finding supported the explanation of differential reporting in the telephone interview.

The odds ratios for selected categories of birth defects calculated from both the telephone interview and hospital records study are presented in Table 7-45. The rate of birth defects increased for both cohorts when malformations were identified in the medical records.

The extent of differential reporting between the two cohorts has also been described (Centers for Disease Control Vietnam Experience Study, 1989). Overall, the authors concluded that agreement between veterans' reports and hospital records for the presence of a birth defect was "relatively poor" for both cohorts. Positive predictive value, sensitivity, and the kappa statistic were slightly lower among Vietnam veterans (Table 7-46). It was further stated that there was no evidence of selection bias or participation bias in this substudy because no differences were noted between cohorts in health histories and demographic or military covariates, and the participation of both groups of veterans was high. However, the subjects in this substudy were selected from the group of veterans who completed the

Table 7-45. Odds Ratios for Selected Categories of Birth Defects for the Telephone Interview and Hospital Records Study in the Vietnam Experience Study, 1989

	Telephone Interview			
Defect Category	Rate (per 1,000) Vietnam veterans	Rate (per 1,000) Controls	OR	95% CI
Total	64.6	49.5	1.3	1.2-1.4
	Hospital Records Study			
Total	72.6	71.1	1.0	0.8-1.4
Major	28.5	23.5	1.1	0.7-1.8
Minor	32.4	34.3	1.00	0.7-1.5

Adapted from the Centers for Disease Control Vietnam Experience Study (1988d).

Table 7-46. Results of the Misclassification Analyses for Birth Defects in the Hospital Records Substudy, Vietnam Experience Study, 1989

Vietnam veterans		Non-Vietnam veterans	
PPV:	24.8%	PPV:	32.9%
NPV:	95.2%	NPV:	95.8%
Sensitivity:	27.1%	Sensitivity:	30.3%
Specificity:	94.7%	Specificity:	96.2%
% Agreement:	90.6%	% Agreement:	92.4%
Kappa index:	20.9%	Kappa index:	27.6%

PPV = Positive predictive value.
NPV = Negative predictive value.
Adapted from the Centers for Disease Control Vietnam Experience Study (1989).

examination. The response rate for this phase of the study was not high, as noted above, with 75% of Vietnam veterans and only 63% of the non-Vietnam veterans participating in the medical examination. While characteristics of the subset who agreed to undergo physical examination did not differ from the telephone interview sample, no data on those who refused the exam and no reasons for refusing to participate were provided in this paper.

Adjusted odds ratios for two additional outcomes verified in the hospital records substudy, low birth weight (AOR=1.1, 95% CI=0.8-1.4) and perinatal mortality (AOR=1.6, 95% CI=0.8-3.1), did not differ by Vietnam service status. A race-specific analysis of total, major, and minor defects showed an increased risk for total and minor birth defects among black veterans. The adjusted odds ratio was 3.3 (95% CI=1.5-7.5) for total defects and 2.9 (95% CI=1.1-8.0) for minor malformations. This finding is based on very small numbers, however, and on multiple occurrences of two minor defects in two families.

From data obtained during the telephone interview, the adjusted odds ratio for Vietnam service and spontaneous abortion was 1.3 (95% CI=1.2-1.4). Although an excess among Vietnam veterans was noted across all three trimesters, only the association in the first trimester was significant. There was no attempt to confirm this end point by using hospital records. No significant differences were observed for the reproductive outcomes of induced abortion (AOR=1.0, 95% CI=0.9-1.2), stillbirths (AOR=0.9, 95% CI=0.7-1.1), ectopic pregnancies (AOR=1.0, 95% CI=0.7-1.2), or childhood cancers (OR=1.5, 95% CI=0.8-2.8).

In addition to the reported excess of total birth defects in the telephone interview, Vietnam veterans also reported more neural tube defects and hydrocephalus than the non-Vietnam veterans. A second substudy was undertaken to examine the increase in cerebrospinal malformations (CSMs). In this substudy, an attempt was made to obtain hospital records for all of the offspring identified in the telephone interview as meeting one of the following criteria: (1) offspring with a CSM as reported by a veteran, (2) those with a reported condition that suggested a CSM, or (3) all reported stillbirths.

Of the 403 offspring reported to have a CSM, 109 were ineligible, 14% (N=58) because of conception prior to father's military service, and 12.6% (N=51) were classified

as miscarriages. Again, the issue of the impact of spontaneous abortion on rates of birth defects is introduced.

The CSM substudy was limited by a poor response rate among the non-Vietnam veterans. The sample thus consisted of 127 offspring of Vietnam veterans (82.5%) and 94 children of the non-Vietnam veterans (67%). Compared with fathers who participated in the CSM study, nonparticipants were more likely to be nonwhite, less educated, unmarried, younger at the time of their child's birth, and have lower general technical test scores. There were also differences in paternal covariates between the participating cohorts that could confound the findings. Compared with Vietnam veterans, non-Vietnam veterans were better educated, had higher general technical test scores, were more likely to be married, were older when their child was born, were less likely to consume alcohol, and were more likely to have had a nontactical primary military occupational specialty (MOS) in the Army, and to have served in both the later and earlier time periods.

The number of CSMs reported by the veterans that were verified by hospital records was examined separately by stillbirth and live birth status. Among the reported stillbirths, five CSMs were documented among children of Vietnam veterans and six among the non-Vietnam veterans. Ten of these 11 CSM had not been reported by the veterans. Positive predictive values derived from this analysis were 6.5% for Vietnam veterans and 8.1% for non-Vietnam veterans.

Among the live births, 21 of 49 reported CSM cases were noted on hospital records for the Vietnam veterans; 6 of the reported 20 cases were observed among the non-Vietnam veterans group, yielding positive predictive values of 42.9% and 30%, respectively.

Tables 35 and 36 in the unpublished technical report of the study list the fathers' descriptions of the birth defects in their offspring obtained through the telephone interview by cohort (Centers for Disease Control Vietnam Experience Study, 1989). It was intriguing to note that among Vietnam veterans, for 22 of the 55 reported cases of birth defects, the hospital record finding was "none." In five additional cases, the hospital record finding was "none of the nervous system." Of these 27 nondocumented reports of birth defects, 3 cases were reported as having died within the first year of life. Death during the first year of life was also reported for one of the eight unverified CSMs in the non-Vietnam veterans cohort.

In summary, the CDC investigators concluded that for "most reproductive and child health outcomes studied, Vietnam veterans were more likely to report an adverse event than were non-Vietnam veterans." In the two substudies conducted to compare rates that were identified through hospital records, no significant differences in adverse outcomes between the two cohorts were determined. However, the ability of this study to address the issue of 2,3,7,8-TCDD exposure and reproductive outcome is severely limited. The question of bias still remains in the two substudies. In addition, exposure was defined as service in Vietnam, which does not provide much insight into the question of the reproductive toxicity of 2,3,7,8-TCDD.

7.13.15.3. *American Legion Study*

The relation of self-reported exposure to Agent Orange and reproductive outcomes was part of a study conducted among 6,810 American Legionnaires who had served during the Vietnam war (Stellman et al., 1988). Information was obtained through a questionnaire mailed to 2,858 veterans (42%) who had served in Southeast Asia and 3,933 veterans (58%) who had served elsewhere. No association between Agent Orange exposure and difficulty with conception, time to conception of the first child, or infant birth weight was observed. However, the proportion of spontaneous abortion was significantly higher among the spouses of veterans who served in Vietnam (7.6%) compared with controls (5.5%) ($p<0.001$). These figures were well below the background rate for recognized spontaneous abortion (15-20%) in the general population.

The significance of these findings is limited by the lack of verification of self-reported exposure, the low response rate, the lack of outcome verification through medical records, and the selection of veterans from the American Legion organization, as they may not be representative of all veterans who served during the Vietnam conflict.

7.13.15.4. *Boston Hospital Study*

A case-control study to investigate the relationship between paternal military service in Vietnam and risk of spontaneous abortion was conducted at Boston Hospital for Women (Aschengrau and Monson, 1989). Cases identified through hospital records were

spontaneous abortions ≤27 weeks gestation that occurred between July 1976 and February 1978 (N=201). Paternal identifying information from the hospital birth records was linked with national and state military records to identify those fathers who had served in Vietnam. Frequency of service in Vietnam was compared for cases and all full-term live birth controls (N=1,119) born at the hospital during this time period. No association was detected (OR=0.88, 95% CI=0.42-1.86).

The same investigators conducted a subsequent study that expanded the outcomes examined to include late adverse pregnancy outcomes (Aschengrau and Monson, 1990). Case infants identified through hospital records included 857 congenital malformations, 61 stillbirths, and 48 neonatal deaths that occurred at the hospital during August 1977 and March 1980. "Exposure" was defined by using the same method as in the previous study. Frequency of paternal service in Vietnam was compared for cases and 998 normal term infant controls. Again, no associations with any of these later adverse outcomes were detected (Table 7-43).

7.13.15.5. *The Ranch Hand Study*

7.13.15.5.1. ***Baseline study - 1984.*** This initial report of the health of Ranch Hand personnel used cohort status (Ranch Hand vs. comparisons) as the basis for evaluating effects and exposure. This group of exposed veterans included those who served in Vietnam during 1962-1965, when Herbicides Purple, Pink, and Green were sprayed. These herbicides had higher TCDD concentrations (33 ppm, 66 ppm, and 66 ppm, respectively) than Herbicide Orange with 2 ppm TCDD (Lathrop et al., 1984).

The protocol consisted of a comprehensive personal and family health questionnaire and a physical examination, including an in-depth laboratory analysis. The response rates for each phase of the protocol were quite different both within and between cohorts. Participation in the questionnaire phase was 97% (N=1,174) for the Ranch Hands and 93% (N=956) for controls. In the physical examination phase, participation dropped to 87% (N=1,045) for the Ranch Hands and 76% (N=773) for controls.

Nonresponders were "on the average" younger than participants. Ranch Hand enlisted personnel had higher participation rates than officers, and black Ranch Hand officers

had lower participation rates than nonblack officers. The difference in the response rates for the physical examination phase of the protocol was ascribed partially to the active encouragement of the Ranch Hand Association for participation and the intense media coverage that the study received. The authors stated that the "majority" of reasons given for nonparticipation were "no time-no interest" and passive refusal.

The reproductive outcomes evaluated in this phase of the study were ascertained through questionnaires obtained from both the veterans and their spouses or partners. A total of 7,399 conceptions were analyzed in this report. There were 3,293 conceptions among 1,174 Ranch Hands and 4,106 among the 1,531 controls.

No significant differences were reported for four "measures of fertility": (1) the number of childless marriages, (2) the number of couples having achieved their desired family size, (3) the number of childless marriages per total number of marriages, and (4) the number of conceptions per years spent together, which included nonmarital relationships. The fertility analysis was performed on the total number of conceptions reported and was not adjusted for any confounding variables. Moreover, exposure in this analysis was defined by a simple dichotomy of Ranch Hands versus controls.

To examine the relationship between Ranch Hand status and spontaneous and induced abortion, stillbirths, and live births, exposure was stratified by pre- and post-Southeast Asia (SEA) service. The unadjusted analysis indicated that Ranch Hands had increased spontaneous abortion rates in both pre-SEA duty ($p=0.06$) and post-SEA duty ($p=0.13$). The report qualified this by stating that these inferences based on analyses that were not adjusted for "key factors affecting pregnancy outcome are of questionable value," although no similar qualification was given for the fertility analysis. After adjustment for maternal age, smoking, and alcohol use and paternal age, no significant difference was observed for spontaneous abortion.

Among the live births with complete data obtained to allow for adjustment of cofactors, no difference in risk of prematurity was noted. However, the estimate of gestational age was based on parental report, which is not a sensitive measure of gestational length, and it was not clear whether prematurity was analyzed as only a dichotomous variable (<37 weeks, ≥ 37 weeks) or as a continuous variable. No analyses for birth weight

differences by exposure status were performed, which was unfortunate given the controversy regarding the finding of lower birth weight among infants exposed to PCDDs and PCDFs (Yen et al., 1989; Rogan et al., 1988).

Unadjusted analyses were conducted to examine the relationship between exposure and neonatal death, infant death, physical handicaps, birth defects, and learning disabilities. These analyses were stratified by pre- and post-SEA service periods. The results indicated that Ranch Hands were more likely to report physical handicaps ($p=0.07$), birth defects ($p=0.08$), and neonatal deaths ($p=0.02$) in the post-SEA analysis. After adjustment for the maternal and paternal covariates described above, the relationship with birth defects achieved statistical significance ($p=0.04$); the other relationships were not statistically significant.

Twelve of the 76 birth defects reported to have occurred among the Ranch Hands after post-SEA service were skin anomalies (ICD Code 757). When these anomalies are excluded, this relationship is no longer statistically significant ($p=0.14$) although "still of interest."

Finally, semen samples from Ranch Hands ($N=560$) and controls ($N=409$) were analyzed for sperm count and morphology. The response rates for this parameter were 72.5% and 76.5%, respectively, although some of the samples submitted were ineligible for analysis because of prior vasectomies and orchiectomies. Linear regression techniques examined sperm count as a continuous variable and percentages of sperm with abnormal morphology as dependent variables. Independent variables were age and exposure to industrial chemicals. No differences in either parameter were identified.

This finding contrasts with the semen analysis results obtained among 324 Vietnam veterans and 247 non-Vietnam veterans in the Vietnam Experience Study (Centers for Disease Control Vietnam Experience Study, 1988a). That analysis indicated that Vietnam veterans had significantly lower sperm concentrations (OR=2.3, 95% CI=1.2-4.3), below the clinical reference value (20 million cells/mL), than the non-Vietnam veterans. In addition, Vietnam veterans had a significantly lower average proportion of "normal" sperm heads. These analyses were adjusted for six covariates, although industrial chemicals were not among them.

7.13.15.5.2. ***The Ranch Hand study - reproductive outcomes 1992.*** The significant association between Ranch Hand status and birth defects found in the previous study was sufficiently troubling to launch a massive project to verify all reported conceptions and pregnancy outcomes through medical record abstraction. In addition, in 1987 serum 2,3,7,8-TCDD levels were obtained from a subset of Ranch Hands and controls. In 1992, the Air Force released the results of the first study that examined the relationship between direct measure of individual serum 2,3,7,8-TCDD levels and verified reproductive outcomes (Wolfe et al., 1992b). A total of 4,607 conceptions were examined in this study; 2,533 were contributed by 791 Ranch Hands, and 2,074 were contributed by 768 controls.

Ranch Hand personnel were shown to have significantly higher 2,3,7,8-TCDD levels compared with the controls. The median values were 12.8 pg/g and 4.2 pg/g, respectively. The 98th percentile for Ranch Hands was 166.4 pg/g; for controls, 10.4 pg/g. 2,3,7,8-TCDD levels were determined in 1987. These results were used to estimate initial doses received during the veterans' tour in Southeast Asia but not the 2,3,7,8-TCDD level at the time of conception.

The fertility analysis performed in the earlier study was not repeated according to level of serum 2,3,7,8-TCDD, which was a disappointing omission. There was a significant variation in the association between 2,3,7,8-TCDD and miscarriage with time since SEA tour ($\leq$18.6 years or $>$18.6 years) and time of conception (pre- or post-SEA tour) among Ranch Hands with current 2,3,7,8-TCDD levels $>$10 ppt ($p=0.01$) (Table 7-47). This was attributed to the low miscarriage rate among the pre-SEA Ranch Hands with current 2,3,7,8-TCDD levels $>$33.3 pg/g lipids. In examining post-SEA conceptions only, a linear trend can be seen for spontaneous abortions and increasing 2,3,7,8-TCDD levels among Ranch Hands who had "late tours" in SEA, i.e., less than or equal to 18.6 years had elapsed between their tour of duty and current 2,3,7,8-TCDD levels. The opposite trend is noted in Ranch Hands with "early tours," i.e., more than 18.6 years had elapsed between the end of duty and the 1987 blood draw. It was concluded that 2,3,7,8-TCDD did not affect the rates of miscarriage because it seemed "implausible that dioxin would act differently in the two groups."

Table 7-47. Rates of Miscarriage (per 1,000) by Pre- and Post-Vietnam Tour Status and Time Since Tour of Duty, Among 1,475 Ranch Hands with >10 pg/g Serum Dioxin, Ranch Hand Study, 1992[a]

Time of conception	Time since tour (years)	Miscarriage rate (no./n) current dioxin 10-14.9 pg/g	15-33.3 pg/g	>33.3 pg/g	p-Value
Pre-tour	≤18.6	142.0 (23/162)	146.8 (32/218)	48.8 (2/41)	0.014[b]
	>18.6	123.9 (14/113)	159.4 (33/207)	166.7 (16/96)	
Post-tour	≤18.6	92.1 (7/76)	136.6 (22/161)	168.5 (15/89)	
	>18.6	237.3 (14/59)	198.6 (29/146)	121.5 (13/107)	

[a]Adapted from Wolfe et al., 1992b.
[b]Comparison of pre- and post-tour data.

An alternative explanation might be that there is a relationship, but it cannot be detected by this type of analysis. To evaluate the relationship between 2,3,7,8-TCDD level and spontaneous abortion, 2,3,7,8-TCDD level at the time of conception must be considered. Assuming a half-life of 7 years in humans (Pirkle et al., 1989), it would seem reasonable, for example, to assume that the two groups of Ranch Hands with 2,3,7,8-TCDD levels of 10-14.9 pg/g of lipids with post-SEA conceptions may have had very different 2,3,7,8-TCDD levels at the time their children were conceived. This is possible because the early tour veterans had more time to decrease their body burden of 2,3,7,8-TCDD before their bloods were drawn in 1987 than did their late tour counterparts.

Table 7-43 illustrates the risk estimates for reproductive outcomes in the Ranch Hand study. Interestingly, the only statistically significant associations between 2,3,7,8-TCDD and adverse events (total birth defects, genital anomalies, and urinary system anomalies) occurred among Ranch Hands with 2,3,7,8-TCDD levels of 15-33.3 pg/g of lipids and not among those in the >33.3 pg/g group.

The report stated that the "expected dose-pattern" for 2,3,7,8-TCDD and total adverse reproductive outcomes (miscarriage, tubal pregnancy, other noninduced abortive pregnancy, or stillbirth) is the "linear one in which the highest anomaly rate occurs at the highest levels of dioxin." This statement raises at least two questions. If a linear response is assumed, might this imply that very early pregnancy losses occur at the highest 2,3,7,8-TCDD levels, so that the conceptus would not survive long enough to be clinically recognized? Or, are very early pregnancy losses and clinically recognized spontaneous abortions two separate entities with different thresholds? Such a scenario has been suggested to explain changes in spontaneous abortions observed after exposure to radiation in Hiroshima (Miller and Blot, 1972).

These questions are of interest because the rate of each of these end points may directly affect the rates of all subsequent reproductive outcomes that are available for examination. The miscarriages assessed in this study are most likely late spontaneous abortions, as 99.6% of reported miscarriages were verified through medical records.

An analysis in which the 1987 dioxin levels are used to estimate dioxin level at time of conception would be a worthwhile effort. If a relationship between paternal 2,3,7,8-

TCDD level and adverse reproductive outcome does exist, this may help determine the dose-response pattern of the relationship.

No evidence was found to support an association between 2,3,7,8-TCDD and total adverse outcomes. These findings should be viewed with caution in view of the above comments concerning the unexplored area of events early in gestation.

Overall, there was little convincing evidence to support an association between birth weight, examined as both a continuous variable and dichotomized ($<$2,500 g or $\geq$2,500 g), and paternal 2,3,7,8-TCDD level. Analyses that adjusted for covariates included maternal and paternal age, maternal alcohol use and smoking, and race of the father. No assessment of 2,3,7,8-TCDD and prematurity was reported.

The potential association between cohort status and birth defects was examined for all defects combined and 12 additional categories of malformations. The only categories with sufficient numbers of verified post-SEA cases to detect a relative risk of 2 were total birth defects (229 cases among 1,045 Ranch Hands and 289 cases among 1,602 controls) and musculoskeletal deformities (132 cases among Ranch Hands and 180 among controls).

A significant variation was observed in the association between total birth defects ($p=0.03$), defects of the respiratory system ($p=0.03$), and urinary system abnormalities ($p=0.04$) by Ranch Hand versus control status with time of conception (pre- or post-SEA). All of these findings were due to a lower rate among Ranch Hands in the pre-SEA conceptions and a higher rate among the post-SEA conceptions for the Ranch Hands.

Analyses of birth defects by 2,3,7,8-TCDD level did not find any "consistent patterns" to support an association. For example, among children of enlisted flying and enlisted ground personnel, children of Ranch Hands with 2,3,7,8-TCDD levels $\leq$10 pg/g lipids had higher rates (433 per 1,000 and 317 per 1,000) than children of controls with background 2,3,7,8-TCDD levels $<$10 pg/g lipids (229 per 1,000). However, rates of children of enlisted ground personnel with 2,3,7,8-TCDD levels $\geq$33.3 pg/g lipids were not significantly elevated. Again, these analyses were not based on 2,3,7,8-TCDD level at time of conception. Moreover, if the higher 2,3,7,8-TCDD levels were related to early pregnancy loss, these results would make more biological sense, as the abnormal conceptuses due to 2,3,7,8-TCDD exposure would have been lost before the pregnancy was recognized.

There was also a significant association between 2,3,7,8-TCDD levels and neonatal death (OR=5.5, 95% CI=1.5-20.7). Insufficient numbers (N=13) precluded the calculation of an adjusted odds ratio for this finding.

Finally, no association was detected between 2,3,7,8-TCDD level and either sperm count or percentage of abnormal sperm. These analyses were based on semen samples that had been collected in 1982.

7.13.15.6. *Reproductive Hormones*

In laboratory rats, 2,3,7,8-TCDD has been related to decreased testosterone levels with evidence that dioxin decreases testosterone synthesis (Kleeman et al., 1990; Mebus et al., 1987; Moore and Peterson, 1988; Moore et al., 1985).

A reported symptom of men who were exposed to 2,3,7,8-TCDD-contaminated materials as a result of daily exposure and industrial accidents is reduced libido (Baader and Bauer, 1951; Bauer et al., 1961; Suskind et al., 1953). Two independently conducted studies of West Virginia TCP workers noted that exposed study subjects also reported this condition approximately 50% more often than either the unexposed controls or individuals without chloracne (Moses et al., 1984; Suskind and Hertzberg, 1984). Endocrine studies or evaluations of conditions or situations that may lead to a reduction in libido were not conducted.

In the NIOSH study of TCP production workers, questions regarding libido were not asked; however, reproductive hormone levels were measured and related to serum 2,3,7,8-TCDD levels. In linear regression analyses, serum 2,3,7,8-TCDD was positively and significantly related to serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and inversely related to total testosterone after adjustment for potential confounders ($p < 0.05$) (Egeland et al., 1994). The prevalence of abnormally low testosterone was two to four times higher among workers with serum 2,3,7,8-TCDD levels of 20-75 pg/g (OR=3.9, 95% CI=1.3, 11.3), 76-243 pg/g (OR=2.7, 95% CI=0.9, 8.2), or $\geq$244 pg/g (OR=2.1, 95% CI=0.8, 5.8) than among unexposed referents (4.8%) (mean serum 2,3,7,8-TCDD = 7 pg/g). Workers in these same serum 2,3,7,8-TCDD quartiles had a higher prevalence of abnormally high LH than workers with serum 2,3,7,8-TCDD levels of

244 pg/g to 3,400 pg/g, but the differences between each serum 2,3,7,8-TCDD category and referents were not significant.

The Ranch Hand veterans study provides the only other human data available that evaluated the relationship between serum 2,3,7,8-TCDD and testosterone (Roegner et al., 1991). Ranch Hand veterans with current serum dioxin levels exceeding 33.3 pg/g were reported to have a lower mean total serum testosterone level (515.0 ng/dL) than the nonexposed comparison group (525.2 ng/dL), but the difference was statistically nonsignificant. No association was observed with FSH and LH. This same exposure group had a nearly fourfold excess of unspecified testicular abnormalities.

Testosterone, FSH, and LH were also measured in U.S. Army veterans and non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). No significant differences in hormone means were noted between the two groups. Additionally, the proportions of values outside the reference range were also similar.

7.13.15.7. *Comment*

The human data offer some evidence of alterations in male reproductive hormone levels associated with substantial occupational exposure to 2,3,7,8-TCDD. The results support the animal literature in which dioxin-related effects have been observed on the hypothalamic-pituitary-Leydig-cell axis and on testosterone synthesis.

7.14. NONCANCER EFFECTS OF INGESTION OF RICE OIL CONTAMINATED WITH POLYCHLORINATED DIBENZOFURANS, QUATERPHENYLS, AND BIPHENYLS IN JAPAN (YUSHO) AND TAIWAN (YU-CHENG)

This section briefly reviews the noncancer effects observed in Yusho (Japan) and Yu-Cheng (Taiwan) victims, individuals exposed, by ingestion, to large concentrations of compounds structurally related to dioxins, namely polychlorinated dibenzofurans, quaterphenyls, and biphenyls. The history of each incident, the chemicals in question, and levels of exposure are described in this chapter. In addition, other reviews have summarized the numerous papers dedicated to Yusho and Yu-Cheng (Lü and Wong, 1984; Kuratsune, 1989; Rogan, 1989).

Reports describing effects among individuals who ingested the contaminated rice oil both in Taiwan and Japan are limited to acute rather than chronic effects. Studies have not comprehensively evaluated long-term effects despite the facts that almost 25 years have passed since the Yusho incident and 15 years since the Yu-Cheng incident and that serum levels of some contaminants are available for both populations. Recent epidemiologic studies have concentrated on the development of offspring of Yu-Cheng mothers. These children were exposed in utero at the time the contaminants were ingested or were conceived after the poisoning and were exposed to residual contaminants transplacentally or through breast milk (Chen et al., 1992; Guo et al., 1992; Lai et al., 1993; Hsu et al., 1993; Guo et al., 1993).

7.14.1. Acute Effects in Adults and Children Directly Exposed to Contaminated Rice Oil

In both groups, the most notable acute effects are dermatologic and neurologic signs and symptoms of fatigue, headaches, and gastrointestinal distress (nausea, vomiting, abdominal pain) (Kuratsune, 1989; Rogan, 1989).

7.14.1.1. *Yusho*

The initial recognition of Yusho occurred in 1968. As of 1983, a total of 2,060 individuals were identified as part of the Yusho population (Masuda et al., 1985). Five years after exposure ended, the mean concentrations of PCBs in the adipose tissue, liver, and blood of Yusho cases were 1.9 ppm, 0.08 ppm, and 6.7 ppb (Masuda et al., 1985), respectively, which were about twice the levels in the control group. Adipose tissue levels of PCDFs ranged from 6 to 13 ppb (Masuda et al., 1985). Sixteen years after exposure, mean PCQ level in adipose tissue of Yusho cases was 207 ppb, approximately 100 times the level in Japanese controls (Kashimoto et al., 1985).

In addition to the major health effects, other possible outcomes were examined. Effects observed shortly after exposure included elevated triglyceride levels and effects on female reproductive hormones noted by changes in menstrual and basal body temperature patterns and lowered excretion of estrogens and pregnanediol in exposed women (Kuratsune, 1989). However, fertility and other measures of reproductive function were not evaluated.

Evidence of chronic bronchitis and respiratory infections still remained 14 years after exposure ended (Nakanishi et al., 1985). However, more than 10 years postexposure, PCB levels were not related to levels of serum triiodothyronine (T_3), thyroxine (T_4), and thyroxine-binding globulin (TBG) (Murai et al., 1987). Although the liver is the suspected target organ for halogenated hydrocarbons and marked proliferation in the endoplasmic reticulum was observed, clinical evidence of liver damage, such as alterations in liver enzymes or liver disease, was not observed (Kuratsune, 1989).

Dermatologic effects were the most evident signs, characterized by hyperpigmentation of the nails, gingivae, and face and by nail deformities, horny plugs, comedones, acneform eruptions, cysts, and other abnormal keratotic changes (Urabe and Asahi, 1985). Acneform eruptions were observed on the face, cheeks, auricles, retroauricular areas, inguinal regions, and external genitalia (Urabe and Asahi, 1985). More than 80% of Yusho cases experienced one or more dermatologic effects (Kuratsune, 1989), which diminished in severity over time (Urabe and Asahi, 1985).

Ophthalmologic effects were characterized by swelling and hypersecretion of the meibomian glands and pigmentary changes of the conjunctiva (Kuratsune et al., 1972). More than 80% of Yusho cases exhibited ocular changes, which, in some cases, appeared to persist 15 years after exposure ended (Kuratsune, 1989).

Thirty percent of the cases reported having at least one symptom consistent with neurologic involvement, such as limb parasthesia and spasms, weakness, headaches, and fatigue (Kuratsune, 1972). As summarized by Kuratsune (1989), Kuriowa et al. (1969) found mostly sensory deficits, identified through slowed nerve conduction velocities in 23 cases. Follow-up of these cases indicated that the neurologic symptoms disappeared over time; however, conduction velocities were not repeated.

A number of studies examined the immune status of Yusho cases (Kuratsune, 1989). Significant decreases in mean IgA and IgM and increases in IgG were noted in 28 cases tested in 1970 ($p<0.05$) (Nakanishi et al., 1985). Within 2 years, means levels of all three immunoglobulins returned to normal. Small increases in the percentage of CD4 cells, small decreases in the percentage of CD8 cells, and enhanced lymphocyte stimulation were also noted in Yusho cases (Nakanishi et al., 1985).

Studies of offspring of Yusho cases have been limited to descriptions of effects on newborns exposed in utero. An early description of 13 children born to exposed mothers noted two stillborn infants, one of whom was diffusely and deeply hyperpigmented (Rogan, 1982). Neonates described in other reports were darkly pigmented and had marked secretions of the conjunctival palpebra, gingival hyperplasia, hyperkeratosis, calcification of the skull, low birth weight, and natal teeth (Yamashita and Hayashi, 1985). The abnormal pigmentation disappeared after 2 to 5 months. No other physical abnormalities (neurologic, cardiovascular, or malformations) were identified.

7.14.1.2. *Yu-Cheng*

The initial recognition of Yu-Cheng occurred in 1979. As of 1983, approximately 2,000 individuals were found to have been exposed to the contaminated rice oil. Within the first year of exposure, mean serum PCB, PCDF, and PCQ levels for 15 cases were 60 ppm (range 4-188 ppm), 0.14 ppb (range $<$0.005-0.27 ppb), and 19.3 ppb (range 0.9-63.8), respectively (Kashimoto et al., 1985). Analysis of PCB levels in 1980-1981 in 165 cases (mean 38 ppb, range 10-720) (Rogan, 1989) and in 1985 in 32 cases (mean 15.4 ppb, range 0.6-86.8) (Lundgren et al., 1988) suggested that some PCBs were being eliminated. It is not clear from the reports if the samples were drawn from distinctly different individuals or included some of the same individuals.

The ophthalmologic and dermatologic changes observed in Yu-Cheng cases were very similar in character and anatomical distribution to those noted in Yusho cases (Lü and Wu, 1985). In 89 cases followed for up to 17 months, dermatologic conditions of 38% of the cases improved, 54% remained the same, and 7% showed deterioration of their conditions (Lü and Wong, 1984).

Like Yusho cases, Yu-Cheng cases examined within 2 years of exposure for nerve function exhibited slowing of sensory nerve conduction. They also exhibited motor nerve slowing and mixed deficits (Chen et al., 1981, 1983, 1985; Chia and Chu, 1984). Twenty percent of a population of 27 individuals also had abnormal EEGs (Chia and Chu, 1984). However, the authors suggest that any correlation between PCB exposure and the abnormal EEGs may be spurious due to low PCB levels in the cerebrospinal fluid (0.5-2.3 ppb)

(measured in four subjects), despite much higher blood PCB levels of 48-64 ppb. A sample of 28 individuals with peripheral neuropathy in 1980 was reexamined in 1982 and was found to have normal EEGs and some recovery of sensory and motor nerve conduction velocity (Chia and Chu, 1985).

In 1981, immunologic function was assessed on different subsets of Yu-Cheng cases and summarized by Lü and Wong (1984). In 30 cases compared with unexposed controls, both IgA and IgM were significantly decreased, while IgG did not differ from controls. In this same group, percentages of active T cells and T cells were significantly increased ($p<0.05$), while total lymphocyte count and percentage of B cells were unchanged. Significant increases in helper T cells (T4) but not suppressor T cells (T8) were also observed. In another group of cases, response to lymphocyte-stimulating mitogens was mixed and the findings unclear. In 143 cases, reaction to streptococci antigen appeared to be significantly ($p<0.05$) depressed relative to controls.

Alterations in porphyrin levels and liver enzymes have been identified as acute reactions to exposure to halogenated polycyclic hydrocarbons, including PCBs. Porphyrin levels were measured in two exposed groups (Chang et al., 1980; Gladen et al., 1988). In 1980, statistically significant elevations in 24-hour urinary excretion of uroporphyrin (exposed = 41.23 μg $\pm$ 24.56; unexposed = 13.57 μg $\pm$ 11.76, $p<0.01$) and α-aminolevulinic acid (exposed = 1.002 mg $\pm$ 0.600; unexposed = 0.715 $\pm$ 0.337, $p<0.05$) were noted among 69 subjects (Chang et al., 1980). Coproporphyrin and porphobilinogen levels were increased in the exposed group but were not significantly elevated. The second study group was composed of 75 children born between June 1978 and March 1985 to mothers who ingested contaminated rice oil (Gladen et al., 1988). Spot urines were collected in 1985. Mean total porphyrin (exposed = 95.2 μg/L; unexposed = 80.7 μg/L) and coproporphyrin (exposed = 72.4 μ/L; unexposed = 59.8 μ/L) excretion was elevated in the exposed, possibly due to extremely high levels (>200 μg/L) in eight exposed children and two controls (Rogan et al., 1988). However, no porphyria cutanea tarda, a severe form of porphyria, was observed in either group of children. Moderate, but statistically significant, increases were observed in AST and ALT levels in 23 cases tested 1 year after exposure (Lü and Wong, 1984). LDH and bilirubin levels were not significantly elevated. As in Yusho

cases, triglyceride levels were significantly increased by approximately twice the level in unexposed controls.

7.14.1.3. *Effects Observed in Offspring of Yu-Cheng Cases*

A concerted effort has been made to evaluate the overall development of children exposed prenatally to contaminated rice oil ingested by their mothers. Two studies are currently being conducted: one to evaluate children and controls born between 1978 and 1985, and the second, to evaluate children born in 1985 and later. The purpose of the two studies is to determine if there are differences among children exposed in utero by the mothers' ingestion of the contaminated rice oil or shortly afterward and children conceived 6 or more years after direct exposure ended (Chen et al., 1992; Chen et al., 1993).

In terms of musculoskeletal development, several studies have documented delays and abnormalities (Yu et al., 1991; Rogan, 1989; Guo et al., 1993; Chen et al., 1993). In one of the first studies conducted in 1985, Rogan and colleagues (1988) examined 117 children born since the mothers' exposure in 1979 and 107 unexposed controls. In this study, babies of exposed mothers were consistently smaller and shorter at birth than controls and had similar characteristics: natal teeth, neonatal conjunctivitis, and pigmentation. Exposed mothers reported a mean birth weight 479 g lower than that reported by control mothers; no validation of these reports using medical records was undertaken. As older children, they exhibited a variety of signs and symptoms: fragile chipped teeth and gum hypertrophy, pigmented and deformed fingernails and toenails, and abnormal lung auscultation. In this same study, neurologic developmental assessments were also conducted to evaluate development (Yu et al., 1991). Forty-nine percent of Yu-Cheng children compared with 22% of controls were developmentally delayed in 32 of 33 developmental milestones, 12% had clinical evidence of developmental or psychomotor delays compared with 2% of controls, and 7% of Yu-Cheng children versus 3% of controls had speech problems. These delays were noted at all ages and persisted over 2 years of testing. Delay was greater in children of smaller size and in children who had exhibited neonatal symptoms of intoxication.

In 1991, the musculoskeletal development of 56 Yu-Cheng children (age range 6-10 years) and their matched controls was again assessed. Only children born first after the

mother's exposure were shorter in stature and had decreased lean muscle mass and soft tissue content (Guo et al., 1992). A subsequent examination of 110 Yu-Cheng children and 108 controls, ages 8 through 14 years, found Yu-Cheng girls to be significantly shorter than controls, matched to the exposed children by age, sex, maternal age, parents' combined educational level, occupation, and neighborhood (Guo et al., 1993). As measured by the Tanner scale, sexual maturation was not slower in Yu-Cheng boys or girls. However, Yu-Cheng boys aged 11-14 had significantly shorter penis length, but testicular and scrotal development did not differ from the controls. Penile length was not related to sexual development as measured by the Tanner scale.

With a validated and standard battery of tests, cognitive and behavioral development of Yu-Cheng offspring was studied yearly from 1985 through 1991. Throughout the testing period, Yu-Cheng children scored consistently lower in the Standford Binet IQ (SB-IQ) and 4-5 points lower than controls (with the same matching criteria as the above study) in three subscales of the Wechsler Intelligence Scale for Children, Revised (WISC-R): verbal IQ (VIQ), performance IQ (PIQ), and full-scale IQ (FIQ) (Chen et al., 1992; Lai et al., 1993). Yu-Cheng children are also reported to exhibit more health, habit, and behavior problems as reported by parents responding to the Rutter's scale and to manifest higher activity based on teachers' responses to the Teacher's Activity Check List (Hsu et al., 1993).

Analysis of physical and cognitive development began in October 1991 of 104 children whose mothers were exposed and 109 children whose fathers but not mothers were exposed and of three matched controls born after 1985 (Guo et al., 1993). Like children born before 1985, the later-born children were shorter in stature and lower in weight than controls, although the authors indicate that the differences have disappeared. Yu-Cheng children are reported to have higher activity levels but do not have temperament, physical, habit, or behavioral problems. Overall, scores on all tests in paternally exposed children were similar to those of the controls. However, maternally exposed children scored lower on the SB-IQ and on all subscales of the WISC-R.

7.14.1.4. ***Comment***

Data from Yusho and Yu-Cheng strongly implicate direct ingestion of contaminated rice oil with numerous acute effects of the skin and peripheral and central nervous systems. The data on immunologic function suggest possible effects, but the numbers of subjects in the various studies were too small to determine an exposure-response relationship. Similarly, data on elevated triglyceride and liver enzyme levels are from a small number of cases and, therefore, the relationship between exposure and the effect is unclear. Furthermore, there are few data to evaluate the long-term effects of these very high exposures. Because many of the 4,000 individuals who were exposed in these two episodes were children, longitudinal studies would be invaluable in assessing the long-term health effects of these exposures.

One difficulty in evaluating the various reports relating to Yusho and Yu-Cheng is the inability to determine if the effects are generalizable to the entire exposed population. It is the impression of this reviewer that many of the cases reported in the literature were those that exhibited the severest signs and symptoms and probably had the highest body burden of contaminants. Some reports included small numbers of cases and controls, relative to the size of the exposed population, and others had no controls. Finally, while much work has gone into determining the severe acute effect, it would be interesting to know what chronic, age, and gender-specific effects are now being exhibited in the approximately 4,000 individuals directly exposed to the contaminated rice oil.

Another difficulty is the presence of several chlorinated hydrocarbons in the contaminated oil, which results in uncertainty as to which contaminant or combination of contaminants is responsible for the noted effects. The data on the offspring of exposed Yu-Cheng mothers and fathers are fascinating and disturbing. It is apparent that parental exposure--more specifically, maternal exposure--to PCBs, PCDFs, and PCQs is directly linked to in utero exposure of the fetus, affecting cognitive, selective musculoskeletal, and possibly, sexual development of the offspring. The ongoing assessment of development in the Yu-Cheng children is imperative to determine the long-term consequences of these exposures on the children's future quality of life and to emphasize the lesson that parental exposures greatly affect the fetus. Prospective studies of fertility and reproductive

experience in these offspring would provide insight into possible intergenerational effects of exposure to these compounds.

7.15. SUMMARY

The data presented in this chapter describe nonmalignant effects in epidemiologic studies of populations with the potential for exposure to chemicals contaminated with 2,3,7,8-TCDD. The purpose of this review is to highlight the salient results of the studies and to assess whether the observed effect was related to exposure to 2,3,7,8-TCDD.

In summary, based on the results of two or more studies, recent evidence suggests that chloracne, elevated GGT levels, an increased risk of diabetes, and altered reproductive hormone levels (luteinizing hormone, follicle-stimulating hormone, and testosterone) appear to be long-term consequences of exposure to 2,3,7,8-TCDD (Table 7-48). In contrast, multiple studies show possible acute effects but few chronic exposure-related effects for dermatologic end points other than chloracne, such as eye lid cyst, hypertrichosis, hyperpigmentation, actinic keratosis, and Peyronie's disease; for liver diseases such as cirrhosis, liver enlargement, and hepatic enzyme levels (LDH, AST, ALT, and D-glucaric acid) other than GGT; and for porphyrias and renal, neurologic, and pulmonary disorders. Circulatory and heart diseases, reproductive outcomes, immunologic disorders, lipid levels, and thyroid function require further study before their respective relationships to 2,3,7,8-TCDD can be more definitively assessed. Studies are under way in several locations that may yield useful information regarding these outcomes.

In the best of circumstances when reviewing studies, it would be ideal if all studies examined the same end points in the same manner, had sufficient statistical power to detect truly positive findings, had good estimates of extent of exposure, and had consistent exposure-response relationships. In the absence of ideal situations, epidemiologists examine the evidence of studies using "six tenets of judgment" (Hatch and Stein, 1986; Hill, 1965) to assess the collective wisdom of the study results. These tenets are: temporality (sequence of events), degree of exposure, strength, consistency and specificity of association, and biologic plausibility.

Table 7-48. Summary of Effects Observed in Humans

System	Acute	Chronic
Dermatologic	conjunctivitis red and irritated eyes blepharitis	chloracne
Liver	temporary enlargement	--[a]
Liver enzymes	↑ GGT ↑ AST ↑ ALT ↑ D-glucaric acid excretion	↑ GGT
Urinary porphyrins	+/-[b] PCT uroporphyrin urobilinogen coproporphyrin	--
Lipids	↑	--
Cholesterol	↑	--
Triglycerides	↑	--
Other GI	+\- RUQ[c] pain loss of appetite nausea	--
Thyroid	↑ T_4 ↑ T_4/TBG in some studies	--
Diabetes	no data	+/-
Immune	no data	+/- ↑NK cells +/- ↑IgA
Neuro	+/- ↓ libido ↑ irritability ↑ nervousness ↓ pin prick sensation	--
Circulatory	--	--
Pulmonary	irritation	--
Renal	--	--
Reproductive	--	+/-
Reproductive hormones	no data	↑ LH in ♂ ↑ FSH in ♂ ↓ testosterone in ♂
Chromosome	+/-	no data

[a]No effect noted.
[b]Some positive and some negative studies.
[c]Right upper quadrant.

In evaluating many of the studies that examined the relationship between serum 2,3,7,8-TCDD, there are several common threads that bear noting. They will be discussed first to avoid repetition throughout the summary.

In terms of temporality, all studies reviewed in this chapter were conducted after the presumed exposure occurred. Some of the studies were completed shortly after the exposure, as in Seveso (Mocarelli et al., 1986; Filippini et al., 1981); others were conducted many years after the groups' last exposure to evaluate the more chronic health outcome (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1987; Roegner et al., 1991; Assennato et al., 1989; Calvert et al., 1991, 1992, 1993; Alderfer et al., 1992; Sweeney et al., 1990; Egeland et al., 1994; Sweeney et al., 1992; Webb et al., 1989; Ott et al., 1993b). One dilemma in assessing the effect of past exposures is ascertaining whether an effect observed many years postexposure is due to the exposure itself or to an exposure or event that occurred during the intervening period. Another problem is determining what, in the analysis, the investigator considered the most important of the possible confounding exposures. Finally, restricting examination of events to those that occurred after the exposure does not in and of itself satisfy this time order criterion. Several factors must be considered, such as the half-life of the contaminant in the body and the concentration at the time of the event. Consistency in the results of similarly designed studies of 2,3,7,8-TCDD-exposed populations should help strengthen the conclusion of an effect or no effect.

Determination of the extent of exposure throughout the studies was varied. When the risk of disease increases with the dose or gradient of exposure, the evidence for causation is strengthened. It should be emphasized that there are many possible dose-response patterns, which may result in different threshold levels for different end points. Due to the exposure misclassification bias present in most of the dioxin research, with the exception of a few studies, it is not valid to attempt to determine dose-response relationships. To summarize, four studies evaluated the relationship between nonmalignant effects and body levels of 2,3,7,8-TCDD: the Ranch Hand study of U.S. Air Force personnel (Roegner et al., 1991); the study of 50 Missouri residents (Webb et al., 1989); the evaluation of the BASF accident cohort (Ott et al., 1993b); and the NIOSH study of 281 TCP production workers (Sweeney et al., 1989, 1990, 1993; Calvert et al., 1991, 1992, 1993; Alderfer et al., 1992; Egeland et

al., 1994). In 1988, the workers in the NIOSH study had the highest serum levels (mean= 220 pg/g) in these four studies.

In two populations, Seveso and U.S. Army veterans (Mocarelli et al., 1991; Centers for Disease Control Veterans Health Studies, 1988), serum 2,3,7,8-TCDD levels were measured, but the levels were not used to examine dose-response relationships. In the Veterans Health Studies, more than 99% of the serum 2,3,7,8-TCDD levels of the sample of both Vietnam and non-Vietnam veterans were at the background level (4 pg/g). Therefore, comparisons were made between the two groups as a whole.

Serum levels of Seveso residents were obtained for a small proportion (N=20) of the total number of residents of Zone A within 1 year of the reactor release (Mocarelli et al., 1991). The data suggest that the levels may be related to a number of factors, including age (younger children were outside at the time of the release), whether the resident was inside or outside, ingestion of local produce, number of days of residence in the area after the release, to name a few. The data suggest that the potential for substantial exposure was high for individuals residing in the area. The range of levels in the 20 Zone A residents was 820 pg/g to 56,000 pg/g (median = 7,400 pg/g).

The majority of the remaining studies examined the differences between individuals identified as exposed or unexposed or with or without chloracne. Most of these studies did not evaluate other parameters that might explain differences in effects between exposed and unexposed, for example, the length of exposure. However, one study assessed dose-response relationships based on a statistical algorithm of intensity and highest dose of TCDD exposure (Bond et al., 1989).

In terms of the magnitude (or strength) of the association, this criterion refers to the degree to which the measure of association (e.g., odds ratio or relative risk) exceeds the null value of 1. The stronger the association between exposure and effect, the more convincing is the argument for causation. There is no definitive cutpoint to numerically define a meaningful measure of association. Other factors, such as the prevalence of the exposure in the population, affect the significance of the measure. Because so many adverse health conditions are multifactorial in etiology, a general rule of thumb is that a relative risk less than 2 renders a cause-effect relationship less likely.

A critical element that should always accompany the effect measure is a confidence interval. Placement of an interval around the measure enables quantitation of the result for a more meaningful interpretation. An odds ratio of 30 is quite impressive, but if the 95% confidence interval is 0.9-200, the magnitude of the association is less impressive.

If an association between a factor and a disease is demonstrated across a variety of studies employing different designs and different populations (consistency), then the argument for causation is strengthened. Replication of an association under different conditions decreases the likelihood that confounding is responsible for the observed association. Consistency is a powerful criterion for causation but only when "the variables under test (exposure, outcomes) are similar enough" to justify the comparison of the various studies' findings (Hoffman et al., 1986).

It should also be determined a priori that each study included in the critical evaluation process is in adherence to basic epidemiologic principles governing study design and analysis. Deficient studies with suspect results should be excluded. While this is not to imply that such studies have no worth, as invaluable information has often been derived from these studies that improve on subsequent examinations of the issue, they have no place in the evaluation process. Unfortunately, in studies of 2,3,7,8-TCDD and effects in humans, the probability of exposure misclassification forces exclusion of much of the research to date.

Specificity refers to the uniqueness of the association between a factor and an outcome. If the relationship were absolute, then only factor X would be related to only effect Y. It is indeed rare to encounter this type of association, which renders this criterion generally less useful in the evaluation process.

Finally, according to the criterion of biological plausibility, the observed association between exposure and effect should be consistent with existing theory and information from other scientific disciplines. Certainly one would feel more secure in the causation debate if the biological basis for an observed association can be explained. However, biological implausibility may simply reflect gaps in existing scientific knowledge that could explain the relationship.

7.15.1. Effects Having a Positive Relationship With Exposure to 2,3,7,8-TCDD

The following section describes those end points for which there is good evidence from two or more studies suggesting an effect of exposure to 2,3,7,8-TCDD.

7.15.1.1. *Chloracne*

7.15.1.1.1. ***Temporality.*** Chloracne is one of the best known of the medical consequences of exposure to 2,3,7,8-TCDD-contaminated substances. In general, it has been observed in most incidents where substantial exposure has occurred, particularly among TCP production workers (Goldman, 1972; May, 1973; Bleiberg et al., 1964; Bond et al., 1987; Suskind and Hertzberg, 1984; Moses et al., 1984; Zober et al., 1990) and Seveso residents (Reggiani, 1978; Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). As previously stated, chloracne appears within several weeks to months from the time of exposure, often resolving after discontinuation of exposure (Moses et al., 1984; Suskind and Hertzberg, 1984), although for some it may remain for extended periods after exposure ended (Moses et al., 1984).

7.15.1.1.2. ***Degree of exposure, consistency of the association.*** The amount of exposure necessary for development of chloracne has not been resolved, but studies suggest that high exposure (both high acute and long-term exposure) to 2,3,7,8-TCDD increases the likelihood of chloracne, as evidenced by chloracne in TCP production workers and Seveso residents who have documented high serum 2,3,7,8-TCDD levels (Beck et al., 1989; Fingerhut et al., 1991a; Mocarelli et al., 1991; Neuberger et al., 1991) or in individuals who have a work history with long duration of exposure to 2,3,7,8-TCDD-contaminated chemicals (Bond et al., 1989). The absence of substantial chloracne in U.S. Army Vietnam veterans whose mean serum 2,3,7,8-TCDD levels were at background (4 pg/g) (Centers for Disease Control Vietnam Experience Study, 1988d) and U.S. Air Force Ranch Hands whose serum 2,3,7,8-TCDD levels fell intermediate to those of workers and Army Vietnam veterans (Roegner et al., 1991) suggests that there is a higher incidence of the disorder among those with higher serum 2,3,7,8-TCDD levels.

7.15.1.1.3. ***Strength of the association.*** In earlier studies, chloracne was considered to be a "hallmark of dioxin intoxication" (Suskind, 1985). However, only in two studies were risk estimates calculated for chloracne. Both were studies of different cohorts of TCP production workers (Suskind and Hertzberg, 1984; Bond et al., 1989); one group was employed in a West Virginia plant, the other in a plant in Michigan. Of the 203 West Virginia workers, 52.7% ($p<0.001$) were found to have clinical evidence of chloracne, and 86.3% reported a history of chloracne ($p<0.001$) (Suskind and Hertzberg, 1984). None of the unexposed workers had clinical evidence or reported a history of chloracne. Among the Michigan workers, the relative risk for cases of chloracne was highest for individuals with the longest duration of exposure ($\geq$60 months; RR=3.5, 95% CI=2.3-5.1), those with the highest cumulative dose of TCDD (based on duration of assignment across and within 2,3,7,8-TCDD-contaminated areas in the plant) (RR=8.0, 95% CI=4.2-15.3), and those with the highest intensity of 2,3,7,8-TCDD exposure (RR=71.5, 95% CI=32.1-159.2) (Bond et al., 1989).

7.15.1.1.4. ***Specificity of the association.*** Chloracne is associated with exposure to other polyhalogenated chemicals, including dibenzofurans, PCBs, naphthalenes, and others (Taylor, 1979). The likelihood of exposure to other polyhalogenated chemicals in the populations studied is probably low, particularly among the Seveso children, whose exposure was to TCP reactant effluents that were primarily contaminated with 2,3,7,8-TCDD. The issue is more relevant in chemical workers, who by virtue of their occupation, have the potential for exposure to other chemicals. Yet, much of the documented chloracne appeared shortly after TCP reactor releases (Ashe and Suskind, 1950; Goldman, 1972; May, 1973) or during TCP or 2,4,5-T production (Bond et al., 1989), suggesting that 2,3,7,8-TCDD was the chloranegenic agent.

7.15.1.1.5. ***Biologic plausibility.*** Animal studies have been effective in describing the relationship between 2,3,7,8-TCDD and chloracne, particularly in rhesus monkeys (McNulty, 1977; Allen et al., 1977; McConnell et al., 1978). Subsequent to exposure to 2,3,7,8-TCDD, monkeys developed chloracne and swelling of the meibomian gland, a modified

sebaceous gland. The histologic changes in the meibomian gland are physiologically similar to those observed in human chloracne (Dunagin, 1984).

In summary, the evidence provided by the various studies convincingly states what is already presumed, that chloracne is a common sequela of exposure to 2,3,7,8-TCDD. More information is needed to determine the level and frequency of 2,3,7,8-TCDD exposure needed to cause chloracne and whether personal susceptibility plays a role in the etiology. Finally, it is important to recall that the absence of chloracne does not imply lack of exposure (Mocarelli et al., 1991).

7.15.1.2. *Gamma Glutamyl Transferase (GGT) Levels*

7.15.1.2.1. ***Temporality, degree of exposure, and strength and consistency of association.*** There appears to be a consistent pattern of increased GGT levels among individuals exposed to 2,3,7,8-TCDD-contaminated chemicals. Elevated levels of serum GGT have been observed within a year after exposure in Seveso children (Caramaschi et al., 1981; Mocarelli et al., 1986) and 10 or more years after cessation of exposure among TCP and 2,4,5-T production workers (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992) and among Ranch Hands (Roegner et al., 1991). All of these groups had a high likelihood of substantial exposure to 2,3,7,8-TCDD. In addition, for those studies that evaluated dose-response relationships with 2,3,7,8-TCDD levels, the effect was observed only at the highest levels or categories of 2,3,7,8-TCDD.

In contrast, although background levels of serum 2,3,7,8-TCDD suggested minimal exposure to Army Vietnam veterans, GGT was increased, at borderline significance, among Vietnam veterans compared to non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). In addition, despite the increases observed in some occupational cohorts, other studies of TCP production workers from West Virginia or Missouri residents measured but did not report elevations in GGT levels (Suskind and Hertzberg, 1984; Webb et al., 1989).

7.15.1.2.2. ***Specificity.*** In clinical practice, GGT is often measured because it is elevated in almost all hepatobiliary diseases and is used as a marker for alcoholic intake (Guzelian,

1985). In individuals with hepatobiliary disease, elevations in GGT are usually accompanied by increases in other hepatic enzymes, e.g., AST and ALT, and metabolites, e.g., uro- and coproporphyrins. Significant increases in hepatic enzymes other than GGT and metabolic products were not observed in individuals whose GGT levels were elevated 10 or more years after exposure ended, suggesting that the effect may be GGT specific. However, in the Seveso male children and those with chloracne (both sexes), ALT was significantly increased concomitantly with GGT within 1 year of the reactor release (Mocarelli et al., 1986; Caramaschi et al., 1981). In a longitudinal analysis, both enzymes returned to normal levels within 5 years after exposure (Mocarelli et al., 1986; Assennato et al., 1989).

These data suggest that in the absence of increases in other hepatic enzymes, elevations in GGT are associated with exposure to 2,3,7,8-TCDD, particularly among individuals who were exposed to high 2,3,7,8-TCDD levels. The fact that investigators observed a decline in enzyme levels in Seveso children but a continued elevation in TCP workers may reflect differences in how exposure occurred (i.e., acute but high doses in Seveso versus continuous or frequent long-term, medium to high doses in TCP workers) or differences in the metabolism of the maturing versus mature system, or a bit of both.

7.15.1.2.3. ***Biologic plausibility.*** The animal data with respect to 2,3,7,8-TCDD-related effects on GGT are sparse. Statistically significant changes in hepatic enzyme levels, particularly AST, ALT, and ALK, have been observed after exposure to 2,3,7,8-TCDD in rats and hamsters (Gasiewicz et al., 1980; Kociba et al., 1978; Olson et al., 1980). Only one study evaluated GGT levels (Kociba et al., 1978). Moderate but statistically nonsignificant increases were noted in rats fed 0.10 μg/kg 2,3,7,8-TCDD daily for 2 years, and no increases were observed in control animals.

Among humans, increased levels of GGT may suggest activity such as cholestases, liver regeneration, or drug or xenobiotic metabolism. In human adults, 2,3,7,8-TCDD is stored in the adipose tissue and has a half-life of approximately 7 years (Pirkle et al., 1989). Continued GGT activity in adults with serum 2,3,7,8-TCDD levels many times over background levels may reflect continuous, low-level metabolism of 2,3,7,8-TCDD.

In summary, GGT is the only hepatic enzyme found in a number of studies to be chronically elevated in adults exposed to high levels of 2,3,7,8-TCDD. The consistency of the findings in a number of studies suggests that the finding may reflect a true effect of exposure but for which the clinical significance is unclear. Long-term, pathologic consequences of elevated GGT have not been illustrated by excess mortality from liver disorders or cancer or in excess morbidity in the available cross-sectional studies.

It must be recognized that the absence of an effect in a cross-sectional study, for example, liver enzymes, does not obviate the possibility that the enzyme levels may have been increased concurrent to the exposure but declined after cessation. The apparently transient elevations in ALT levels among the Seveso children suggest that hepatic enzyme levels other than GGT may react in this manner to 2,3,7,8-TCDD exposure.

7.15.1.3. *Diabetes and Fasting Serum Glucose Levels*

7.15.1.3.1. ***Strength and consistency of association.*** The epidemiologic evidence for an increased risk of diabetes and for an elevated prevalence of abnormal fasting serum glucose levels is based on two cross-sectional medical studies conducted at least 15 years and up to 37 years after last exposure to 2,3,7,8-TCDD-contaminated chemicals (Sweeney et al., 1992; Wolfe et al., 1992a) and supporting evidence from a third study of the BASF accident cohort (Ott et al., 1993b). All three studies found that individuals at the highest serum 2,3,7,8-TCDD levels had a slight but statistically significant or borderline significant increased risk for developing diabetes or having an elevated fasting serum glucose. Further study is needed to corroborate the findings of these three cross-sectional studies.

7.15.1.3.2. ***Specificity.*** Diabetes mellitus is a heterogeneous disorder that is a consequence of alterations in the number or function of pancreatic beta cells responsible for insulin secretion and carbohydrate metabolism. Depending on its type, diabetes has been attributed to endogenous factors such as genetic predisposition, to autoimmune processes, and to exogenous factors such as viral infections (Yoon et al., 1987) and chemical exposures, notably a rat poison (Miller et al., 1978) and some medications (Wilson and LeDoux, 1989), environmental toxins (Diabetes Epidemiology Research International, 1987), age, obesity,

reduced physical exercise, diet, socioeconomic status, increased insulin resistance by the beta cells, and possibly parity (Pareschi and Tomasi, 1987).

Both the NIOSH and Ranch Hand studies used strict criteria to identify cases. In the NIOSH study, the relationship between exposure to 2,3,7,8-TCDD and possible alterations in glucose metabolism was assessed using two outcome measures: case definition of diabetes and fasting serum glucose levels. Participants met the case definition of diabetes if their fasting serum glucose level was 7.8 mmol/L or greater on two consecutive occasions (National Diabetes Data Group, 1979) or if they reported a positive history of physician-diagnosed diabetes after the date of first employment at the plant. In the Ranch Hand study, diabetic status was assessed by measuring fasting serum glucose and 2-hour postprandial glucose and using a case definition of diabetes. Diabetes was defined as having a verified history of diabetes or an oral glucose tolerance test of ≥ 11.1 mmol/L (200 mg/dL) (Roegner et al., 1991). Both studies used acceptable definitions for epidemiologic studies of diabetes. In the NIOSH study, the definition may exclude some individuals who would have otherwise been classified as diabetic with the glucose tolerance test. However, there should be no differential bias in identifying cases in workers or the unexposed referents.

7.15.1.3.3. ***Biologic plausibility.*** The effects of 2,3,7,8-TCDD on glucose metabolism have been evaluated only in a few laboratory studies (Zinkl et al., 1973; McConnell et al., 1978a; Gasiewicz et al., 1980; Schiller et al., 1986; Ebner et al., 1988; Gorski et al., 1990). Although these studies suggest that 2,3,7,8-TCDD may alter glucose metabolism, for the most part the animal studies do not corroborate the direction of the findings of two cross-sectional medical studies (Wolfe et al., 1992a; Sweeney et al., 1993). Long-term feeding studies to evaluate the relationship between glucose levels, the development of diabetes, and 2,3,7,8-TCDD dose would be helpful in assessing the effect of exposure on the physiologic integrity of the islet cells. In addition, such studies may identify other factors that may affect either directly or indirectly the function of the islet cells.

In summary, both the NIOSH and Ranch Hand studies show that highly 2,3,7,8-TCDD-exposed individuals may be at a slightly greater risk for developing diabetes and

experience a greater prevalence of elevated fasting glucose levels, which may be a precursor of conversion to a diabetic state. However, in the NIOSH study, the traditional risk factors for diabetes--age, body mass index or weight, and family history of diabetes--appear substantially more influential than 2,3,7,8-TCDD in the development of diabetes. These factors could not be evaluated in the data presented in the publicly available material.

7.15.1.4. *Reproductive Hormones*

7.15.1.4.1. ***Strength and consistency of association.*** Levels of reproductive hormones have been measured with respect to exposure to 2,3,7,8-TCDD in three cross-sectional medical studies. Testosterone, LH, and FSH were measured in TCP and 2,4,5-T production workers (Egeland et al., 1994) and in Army Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988d), and testosterone and FSH in Ranch Hands (Roegner et al., 1991). The risk of abnormally low testosterone was two to four times higher in exposed workers with serum 2,3,7,8-TCDD levels above 20 pg/g than in unexposed referents (Egeland et al., 1994). The risk in the Ranch Hands, who had lower exposures than the production workers, was 1.3 times (OR=1.3) that of the comparison group (Roegner et al., 1991). No significant associations were found between Vietnam experience and altered reproductive hormone levels (Centers for Disease Control Vietnam Experience Study, 1988d). Only the NIOSH study found an association between serum 2,3,7,8-TCDD level and increases in serum LH.

7.15.1.4.2. ***Specificity.*** The NIOSH study excluded from analysis participants who had conditions that might have influenced gonadotropin and/or testosterone levels: history of prostate cancer, thyroid or other hormone usage, or liver cirrhosis. Similarly, in the Ranch Hand study, individuals with orchiectomies or who were taking testosterone medication were excluded from the analysis of testosterone; no participants were excluded from the analyses of FSH. The CDC study of Vietnam veterans did not describe the exclusions.

7.15.1.4.3. ***Biologic plausibility.*** In rats, 2,3,7,8-TCDD has been shown to decrease testosterone levels (Moore et al., 1985; Moore and Peterson, 1988; Mebus et al., 1987)

through a decrease in testosterone synthesis (Kleeman et al., 1990) or by decreasing the production of pregnenolone from cholesterol (Ruangwies et al., 1991). In addition, 2,3,7,8-TCDD has been shown in rats to reduce the responsiveness of the pituitary to testosterone (Bookstaff et al., 1990a) and of the Leydig cells to LH stimulation (Moore et al., 1991).

The findings of the NIOSH and Ranch Hand studies are plausible given the pharmacological and toxicological properties of 2,3,7,8-TCDD. A mechanism responsible for the effects may involve the ability of 2,3,7,8-TCDD to influence hormone receptors. The aromatic hydrocarbon (Ah) receptor to which 2,3,7,8-TCDD binds resembles steroid hormone receptors in both structure and mode of action. Studies suggest that 2,3,7,8-TCDD modulates hormone receptors, including estrogens (Romkes and Safe, 1988; Romkes et al., 1987), prolactin, and its own Ah receptor (Poland and Glover, 1980; Morrow et al., 1986). However, the effect of 2,3,7,8-TCDD on testosterone receptors has not been evaluated.

In summary, the results from both the NIOSH and Ranch Hand studies are limited by the cross-sectional nature of the data and the type of clinical assessments conducted. However, the available data provide evidence that alterations in human male reproductive hormone levels are associated with serum 2,3,7,8-TCDD.

7.15.2. Possible Acute Effects of Exposure to 2,3,7,8-TCDD

The following section reviews end points that were described in groups shortly after exposure to 2,3,7,8-TCDD but were not observed as chronic effects in studies conducted many years after exposure ceased. Also reviewed are end points observed as long-term effects in single studies.

7.15.2.1. *Dermatologic Conditions Other Than Chloracne*

Dermatologic conditions other than chloracne, such as hyperpigmentation, hypertrichosis, and eyelid cysts, have been related to exposure to 2,3,7,8-TCDD in early case reports (Ashe and Suskind, 1950; Suskind et al., 1953; Bleiberg et al., 1964; Poland et al., 1971; Bauer et al., 1961; Goldman, 1972; Jirasek et al., 1974; Oliver, 1975). However, these conditions may have been acute effects of 2,3,7,8-TCDD exposure that resolved over time or may be residual effects of chloracne because they appear to occur more frequently in

individuals with persistent chloracne (Suskind and Hertzberg, 1984). These conditions were not observed in studies in which the cohorts were examined years after cessation of exposure, in individuals with the potential for high exposure, or in those with high adipose or serum 2,3,7,8-TCDD levels (Moses et al., 1984; Webb, 1989; Roegner et al., 1991).

Actinic keratosis, Peyronie's disease, and basal cell carcinoma may not be associated with 2,3,7,8-TCDD. All three conditions were observed in only one study (Suskind and Hertzberg, 1984; Lathrop et al., 1984) and were not observed in studies of individuals with similar potential for exposure (Ott et al., 1993b).

7.15.2.2. *Liver Enzymes Other Than GGT and Hepatomegaly*

A number of studies reported elevated liver enzymes, particularly AST and ALT, among individuals who were being exposed at the time of the measurement (May, 1973) or whose exposure was within a few years of the measurement (Jirasek et al., 1974; Mocarelli et al., 1986; Caramaschi et al., 1981). Follow-up studies or longitudinal analyses of exposed cohorts suggest that the increase in enzyme level resolves over time (Mocarelli et al., 1986; Assennato et al., 1989; Pazderova-Vejlupkova et al., 1981; May, 1982). In studies of exposed populations tested many years after exposure ceased, levels of AST and ALT were within normal range (Calvert et al., 1992; Webb et al., 1989; Roegner et al., 1991; Suskind and Hertzberg, 1984; Moses et al., 1984).

D-glucaric acid was tested in a number of 2,3,7,8-TCDD-exposed populations as an indicator of enzyme induction (Ideo et al., 1985; Martin, 1984; Roegner et al., 1991; Calvert et al., 1992). Shortly after the TCP reactor release, D-glucaric acid levels in Seveso children were elevated (Ideo et al., 1985). No other studies of exposed groups tested 5 to 37 years after exposure ceased found elevations of this enzyme (Martin, 1984; Roegner et al., 1991; Calvert et al., 1992).

These data suggest that certain hepatic enzymes are increased as a response to high, exogenous exposure to 2,3,7,8-TCDD. Once the exposure ends, the enzyme levels seem to decrease over time, as observed in the Seveso populations (Mocarelli et al., 1986; Ideo et al., 1985). Additional evidence of the acute nature of AST, ALT, and D-glucaric acid elevations is demonstrated by the lack of such increases in studies of highly exposed groups

conducted long after exposure ceased (Calvert et al., 1992; Roegner et al., 1991; Martin, 1984).

As in the case of ALT, AST, and D-glucaric acid, hepatomegaly appears to be a condition reported in case reports after high exposure to 2,3,7,8-TCDD-contaminated chemicals, particularly among TCP production workers examined after a TCP reactor explosion and among Seveso residents (Ashe and Suskind, 1950; Suskind et al., 1953; Jirasek et al., 1974; Reggiani, 1980). Later studies conducted after exposure ceased failed to find excess dose-related hepatomegaly in the exposed populations (Bond et al., 1983; Suskind and Hertzberg, 1984; Moses et al., 1984; Calvert et al., 1992; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Webb et al., 1989; Hoffman et al., 1986). However, the absence of an effect in cross-sectional studies does not confirm the lack of an effect in the past.

7.15.2.3. *Pulmonary Disorders*

Early case reports suggest that exposure to 2,3,7,8-TCDD chemicals may cause temporary respiratory irritation (Zack and Suskind, 1980) and tracheobronchitis (Goldman, 1972). The data from two cross-sectional medical studies provide weak evidence of slightly decreased lung function among exposed individuals (Suskind and Hertzberg, 1984; Roegner et al., 1991). In these studies, the effects may be due more to smoking (Roegner et al., 1991) or to a substantial age difference between the exposed and unexposed groups (Suskind and Hertzberg, 1984). One study of highly exposed TCP production workers found no relationship between serum 2,3,7,8-TCDD levels and chronic obstructive pulmonary disease, bronchitis, or decreased pulmonary function (Calvert et al., 1992).

In conclusion, case reports indicate that intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation. However, the findings from controlled epidemiologic studies conducted many years after exposure do not convincingly support an association between 2,3,7,8-TCDD exposure and chronic effects on the respiratory system.

7.15.2.4. *Neurologic Disorders*

The results of case reports and epidemiologic studies demonstrate that exposure to 2,3,7,8-TCDD-contaminated materials is associated with symptoms referable to the central and peripheral nervous systems shortly following exposure and, in some cases, lasting many years (Filippini et al., 1981; Ashe and Suskind, 1950; Moses et al., 1984). Overall, however, the neurologic status of workers, community residents, and Vietnam veterans exposed to 2,3,7,8-TCDD and evaluated from 5 to 37 years after last exposure appears to be normal (Centers for Disease Control of Vietnam Experience Study, 1988a; Lathrop et al., 1984; Sweeney et al., 1993). The data suggest that, although exposure to 2,3,7,8-TCDD may have been extensive as in exposed workers, Ranch Hands, and Seveso residents, the effects described in case reports may have been transient (Filippini et al., 1981; Lathrop et al., 1984; Centers for Disease Control Vietnam Experience Study, 1988a, b; Assennato et al., 1989; Alderfer et al., 1992; Sweeney et al., 1993). The findings of recent studies suggest that in adults there are no long-term neurologic effects caused by even high exposure to 2,3,7,8-TCDD-contaminated materials, but there is very little information with which to examine the effects of exposure on the developing human neurologic system.

7.15.2.5. *Porphyrias*

In rats and mice, exposure to 2,3,7,8-TCDD has been clearly shown to alter porphyrin metabolism (Goldstein et al., 1973; Smith et al., 1981; Jones and Chelsky, 1986; DeVerneiul et al., 1983; Cantoni et al., 1981; Goldstein et al., 1982). Whether 2,3,7,8-TCDD is associated with porphyrin changes in humans, particularly porphyria cutanea tarda (PCT), is a subject of unresolved debate. It has been suggested that the PCT and elevated urinary porphyrins observed in the New Jersey and Czechoslovakian workers during the years of operation of the plants were the result of exposure to hexachlorobenzene, which was produced at the same time as TCP (Pazderova-Vejlupkova et al., 1981; Jones and Chelsky, 1986). These statements have not been corroborated with strong studies. In the follow-up studies, urinary porphyrin levels of these TCP production workers were not elevated (Pazderova-Vejlupkova et al., 1981; Poland et al., 1971) or did not differ from levels in the

control group (Calvert et al., 1993). Doss et al. (1984) also described transient elevations in coproporphyrins among 22 Seveso residents exposed to 2,3,7,8-TCDD.

Because 2,3,7,8-TCDD is a potent porphyrigen in rats and mice, it has been of interest to determine whether exposures may have contributed to the observed changes in porphyrin levels in human populations. The NIOSH study could not address the question of etiology or transient porphyria, but it did not find porphyria in highly exposed workers many years after their occupational exposure.

7.15.3. Effects For Which Further Research Is Needed

The following section describes end points for which the animal data have demonstrated exposure-related effects, but the human data are inconclusive and require further study.

7.15.3.1. *Diseases of the Circulatory System*

In general, the results of the cohort mortality studies of TCP production workers were remarkably similar. For all of the studies, the standardized mortality ratios for diseases of the circulatory system (ICD-9: ICD 390-459) were approximately 100, meaning that the death rate in the exposed population was nearly the same as that in the general population, controlling for age, race, gender, and calendar year (Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993; Bond et al., 1989; Coggon et al., 1991). None of the SMRs above 100 were statistically significantly elevated.

Mortality from circulatory system diseases among Ranch Hands (SMR=110, 95% CI=60-150) (Michalek et al., 1990) and Australian Vietnam veterans (RR=1.7, 95% CI=0.9-3.0) (Fett, 1987b) was nonsignificantly elevated. There was a deficit of deaths from this cause among U.S. Army Vietnam veterans compared to non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988c). Elevated mortality from circulatory diseases among Seveso residents is considered by the authors to be the result of environmental stresses and possibly other risk factors rather than exposure to 2,3,7,8-TCDD (Bertazzi et al., 1989). In addition, a comparison of Zone A residents (2,3,7,8-TCDD-

contaminated region) with Zone B residents (less contaminated region) found the circulatory disease mortality rate to be higher in the residents of the less contaminated area.

Diseases of the circulatory system, particularly heart disease, are the leading causes of death among populations of most developed nations. Leading risk factors include age, cigarette smoking, elevated lipid levels, obesity, hypertension, diabetes, and physical inactivity (Smith et al., 1992). Among the studies that examined mortality from circulatory system diseases, none directly adjusted SMRs for known risk factors or attempted to evaluate jointly the contribution of known risk factors and 2,3,7,8-TCDD to the mortality rates (Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993; Bond et al., 1989; Coggon et al., 1991). Therefore, given the strong contribution of these risk factors, it is not possible to rule out physical and personal risk factors in the etiology of diseases of the circulatory system and heart in these populations. However, the absence of a "health worker effect" for these causes of death suggests that future research be directed specifically at the relationship between circulatory and heart disease and exposure to 2,3,7,8-TCDD.

Cross-sectional morbidity studies have not found increases in the prevalence of circulatory or heart disease among TCP workers, Ranch Hands, or U.S. Army Vietnam veterans (Suskind and Hertzberg, 1984; Bond et al., 1987; Moses et al., 1984; Centers for Disease Control Vietnam Experience Study, 1988a). In some cross-sectional studies, risk estimates were adjusted for some risk factors, depending on the study (Suskind and Hertzberg, 1984; Poland et al., 1971; Moses et al., 1984; Bond et al., 1983; Centers for Disease Control Vietnam Experience Study 1988a; Roegner et al., 1991). Ranch Hands were the only group to experience marginal differences in diastolic blood pressure, arrhythmias, and peripheral pulse abnormalities after adjusting for selected risk factors (Roegner et al., 1991).

The animal data suggest that at high levels of 2,3,7,8-TCDD, the vascular system, cardiac muscle, and valves and function may be affected by exposure (Kociba et al., 1978; Buu-Hoï et al., 1972; Brewster et al., 1988; Hermansky et al., 1988; Kelling et al., 1987; Canga et al., 1988). However, with the exception of the long-term feeding study (Kociba et

al., 1978), the exposures in animals were single, high doses and the human exposures (except Seveso) were chronic, medium to high doses.

In summary, the animal studies suggest that 2,3,7,8-TCDD causes pathologic changes that may lead to later circulatory system disease. However, long-term studies of mature and aged animals have not been carried out to evaluate these hypotheses and to correlate the results of the animal with the human studies. Few epidemiologic studies were designed to control for many of the risk factors known to cause circulatory system and heart disease, but a consistent absence of the health worker effect for circulatory disorders and heart disease in numerous mortality studies suggests the need for additional research in this area. These studies should also include methods to quantify subject exposure to 2,3,7,8-TCDD.

7.15.3.2. *Reproductive Effects*

7.15.3.2.1. ***Consistency.*** A variety of study designs, including case-control, ecologic, cross-sectional, and historical cohort designs, have addressed the issue of 2,3,7,8-TCDD and reproductive effects in humans. Unfortunately, the different criteria for case definitions across studies make it difficult to compare the results. In addition, the method of case ascertainment for certain end points influences the rate of events observed. The Vietnam Experience Study substudies of veteran-reported birth defects compared with those identified through hospital records demonstrated that rates of self-reported outcomes differed by exposure status. Moreover, predictive value of self-reported events was poor in both cohorts. In contrast, rates of birth defects in the Ranch Hand study were similarly reported by the Ranch Hands and controls. Both groups underreported 7% of birth defects in children conceived prior to their SEA tour and 14% after their tour of duty.

7.15.3.2.2. ***Strength.*** With the exception of the finding that Vietnam veterans were more than twice as likely to have low sperm concentrations (OR=2.3, 95% CI=1.2-4.3), no effect measure greater than 2 was noted in any of these investigations. This is not surprising, given the limitations of the studies, particularly with regard to exposure misclassification. Therefore, the trends across these studies carry more import than "statistically significant" results.

7.15.3.2.3. ***Temporality, dose-response.*** While these studies have restricted inclusion of reproductive events to those that occurred after exposure to 2,3,7,8-TCDD was suspected, no study has evaluated 2,3,7,8-TCDD levels at the time of the outcome. Determination of a 2,3,7,8-TCDD dose-response relationship with adverse reproductive outcomes would not be valid unless individual 2,3,7,8-TCDD levels were available. Elucidation of a dose-response pattern may be derived using data from the Ranch Hand study to calculate 2,3,7,8-TCDD levels at the time of the reproductive event, which would be an important contribution toward understanding this phenomenon. However, with regard to spontaneous abortion, this analysis would not be able to address those losses occurring early in gestation.

7.15.3.2.4. ***Biological plausibility.*** A growing body of animal research described in Chapter 5 lends biological plausibility to the association between dioxin and most of the reproductive end points evaluated in these studies, with the notable exception of molar pregnancies. There is growing evidence that dioxin affects testis and accessory gland weight, testicular morphology, spermatogenesis, and fertility in males. A model for a paternally mediated dioxin effect on congenital malformations has not been reported. Among female animals, the primary reproductive end points that have been examined include decreased fertility and pregnancy loss.

The mechanism by which 2,3,7,8-TCDD causes adverse reproductive and developmental effects has not been well described, although considerable insight has been gained from research focusing on the Ah receptor. While the Ah receptor has been linked with birth defects in several mouse strains, it appears that the mechanism of effect may be dependent on the outcome evaluated, as well as other dioxin congeners to which the population is exposed. Clearly, these relationships in humans have not been adequately investigated.

The discovery in the Times Beach, Missouri; CDC; and Ranch Hand studies that self-reported dioxin exposure and exposure indices developed from the analyses of 2,3,7,8-TCDD in soil and military records to estimate individual exposure are poorly correlated with serum 2,3,7,8-TCDD levels was a critical turning point in understanding the inconsistencies of the research to date regarding 2,3,7,8-TCDD and effects. Thus, because of the likelihood of

exposure misclassification in those studies lacking direct measures of exposure, the findings have been severely limited.

7.15.3.3. *Specific Reproductive End Points*

1. Spontaneous abortions. Miscarriages were investigated in several studies with different designs and varied patterns of parental exposure. Events were generally ascertained by self- or spousal-report. When case ascertainment was through medical records, such as in the Ranch Hand study or the Vietnamese investigations, the events are by definition restricted to those miscarriages that were clinically recognized.

Research in the area of early pregnancy loss indicates that 30-50% of all conceptions are lost prior to or during implantation (Hertig et al., 1959). The rate of loss between implantation and expected first menstrual period ranges from 22% to 30% (Wilcox et al., 1988; Sweeney et al., 1988). Thus it is clear that restriction of the examination of pregnancy loss to those events that are ascertained through self-reports or medical records results in a large proportion of the outcome of interest being missed. In studies of environmental factors and spontaneous abortion in which information is lacking concerning the conditions surrounding conception, "the conflation of different doses with different effects can mislead" (Kline et al., 1989). Because of these discrepancies, it would not be meaningful to pool the results of the research on the association between dioxin exposure and miscarriage to judge the "consistency" of the association.

Overall, it must be acknowledged that the data compiled to date are inadequate to address this issue. To simply enumerate and compare the number of "positive" versus "negative" studies to ascertain consistency in the research would be inappropriate. The reasons for this have been described above in detail, with emphasis on the high (40-50%) exposure misclassification that has been documented in the majority of these investigations, the small sample sizes evaluated, lack of data on dioxin levels at the time of conception, and the unknown impact of early pregnancy loss on identification of birth defects. The animal and human evidence for a 2,3,7,8-TCDD-pregnancy loss relationship is sufficiently suggestive to warrant further investigation. Several studies of various designs and populations have demonstrated weak but consistent associations (Reggiani, 1978; Hatch,

1984b; Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989a; Stellman et al., 1988), whereas others have not (Townsend et al., 1982; Smith et al., 1982; Cutting et al., 1970; Kunstadter, 1982; Report to the Minister for Veterans' Affairs, 1983; Stockbauer et al., 1988; Erickson et al., 1984; Centers for Disease Control Vietnam Experience Study, 1989). (These studies include those that should be restricted to the assessment of military service in Vietnam and reproductive events.) The Ranch Hand study leaves several questions unanswered, including the determination of a dose-response level at the time of conception and adverse reproductive outcomes, as well as the impact of early pregnancy losses on rates of spontaneous abortion and birth defects that survive long enough to be "counted."

2. Congenital malformations. The confusing evidence regarding the relationship between dioxin exposure and birth defects results not only from the same limitations described above for the studies of miscarriage but also from the lack of power to evaluate specific types of malformations. To increase the power to detect a potential relationship, the studies have combined all birth defects together and calculated an odds ratio for total birth defects. Given emerging evidence for etiologic heterogeneity among subgroups of birth defects (Khoury, 1989), it is possible that this approach might dilute the effect measure.

These studies also should be stratified by type of parental exposure, i.e., paternal, maternal, or both. Biologically plausible mechanisms for birth defects resulting from paternal exposure to toxic substances have not been well researched. It has been known for many years that temporary infertility may occur after exposure of human males to certain toxic substances. However, animal research suggests that spermatogenesis is "particularly resilient" after exposure to these chemicals (Pearn, 1983). If dioxin were related to malformations among the offspring conceived after paternal service in Vietnam, it is implied that the effect must occur premeiotically. Some animal studies have found that spermatogonia and spermatocytes (premeiotic spermatogenic cells) were able to repair DNA after exposure to toxic agents, whereas spermatids and spermatozoa did not have this capability (Lee and Dixon, 1978).

A few studies (Hatch, 1984a; Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989a) have suggested an association, including those investigations of the Yusho and Yu Cheng incidents (Rogan, 1982; Yen et al., 1989; Rogan et al., 1988). Many

studies have failed to find a relationship between dioxin and birth defects (Townsend et al., 1982; Smith et al., 1982; Cutting et al., 1970; Kunstadter, 1982; Stockbauer et al., 1988; Erickson et al., 1984; Centers for Disease Control Vietnam Experience Study, 1989; Mastroiacovo et al., 1988). Again, however, in view of the serious limitations of these studies of 2,3,7,8-TCDD and reproductive events, it must be concluded that the relationship between paternal dioxin exposure and congenital malformations remains unknown. However, if military service in Vietnam is the exposure of interest, there is little evidence to support an association with birth defects.

3. "Miscellaneous" end points. Additional reproductive outcomes that were evaluated in a subset of the studies include molar pregnancies (in the Vietnamese studies), infant birth weight, neonatal and infant death, and child cancer and mortality. Mainly because of small sample sizes, it is difficult to reach conclusions regarding neonatal, infant, and child mortality and childhood cancers. However, the increased risk for neonatal death observed in the Ranch Hand study, the only study with individual TCDD levels, should be further investigated. Available evidence does not support an association between paternal dioxin level and low birth weight (Wolfe et al., 1992b; Centers for Disease Control Vietnam Experience Study, 1989); a maternally mediated effect with documentation of TCDD exposure has not been examined.

There was evidence for a maternally mediated effect of dioxin exposure and birth defects in the Vietnamese studies. An attempt to confirm these findings by using recent advances in exposure measurement would be a worthwhile effort.

The Vietnam Experience Study found a significant relationship between service in Vietnam and sperm abnormalities, while the Ranch Hand study did not confirm these results when exposure was defined by both cohort status and 2,3,7,8-TCDD levels. However, the data on alterations in male reproductive hormone levels associated with occupational exposure to 2,3,7,8-TCDD emphasize that further research in these areas is required.

In conclusion, the research to date has been successful in resolving some confusion surrounding the conflicting evidence for an association of dioxin exposure and various reproductive end points in humans. High occurrence of exposure misclassification, differences in case definitions across studies, and small sample sizes have severely limited

the power of these studies to address these questions. Additional research that includes a measure of dioxin level at the time of conception for both the father and mother is necessary if the effect of dioxins on the spectrum of reproductive outcomes is to be understood.

7.15.3.4. *Immunologic Effects*

Information on immunologic function in humans relative to exposure to 2,3,7,8-TCDD is scarce. The available epidemiologic studies do not describe a consistent pattern of effects among the studies. Two studies of German workers, one exposed to 2,3,7,8-TCDD and the other to 2,3,7,8-tetrabrominated dioxin and furan, observed dose-related increases of complements C3 or C4 (Zober et al., 1992; Ott et al., 1993b). Other studies of exposed groups have not examined complement components to any great extent.

More comprehensive evaluations of immunologic function with respect to 2,3,7,8-TCDD exposure are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immunologic system may provide the greatest insight, particularly since animal studies have found significant results in immature animals and human breast milk is a source of 2,3,7,8-TCDD and other related compounds. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, is an immunotoxin in humans.

7.15.3.5. *Lipids*

Animal studies indicate that 2,3,7,8-TCDD is associated with generally increased serum cholesterol and serum triglyceride levels. The effect of exposure to 2,3,7,8-TCDD-contaminated chemicals on lipids is not consistent in the available epidemiology studies. Elevations in total cholesterol and triglyceride levels were reported after high 2,3,7,8-TCDD exposure in TCP workers (Pazderova-Vejlupkova et al., 1981; Martin, 1984) and laboratory workers (Oliver, 1975). Despite their very high exposure to 2,3,7,8-TCDD-contaminated chemicals, neither adults nor children from Seveso had lipid levels above the referent level. Risk factors such as dietary fat intake, familial hypercholesterolemia, alcohol consumption,

and exercise, which also affect cholesterol and other lipid levels, may be factors that were not considered in these studies.

Ranch Hands continue to have elevated lipid levels despite the extended length of time between exposure and testing. In the 1992 phase of the Ranch Hand study, additional parameters that may affect lipid levels were examined. Analysis of the lipid data collected during the NIOSH study also will contribute to the information on lipid levels in relation to serum 2,3,7,8-TCDD levels.

7.15.3.6. *Thyroid Function*

Many effects of 2,3,7,8-TCDD exposure in animals resemble signs of thyroid dysfunction. In the few human studies that examined the relationship between 2,3,7,8-TCDD exposure and thyroid function, the results are mostly equivocal (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Suskind and Hertzberg, 1984). However, TBG appears to be positively correlated with current levels of 2,3,7,8-TCDD in the BASF accident cohort (Ott et al., 1993b). Little information on thyroid function has been reported for production workers and none for Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels. A study of nursing infants suggests that ingestion of breast milk with a higher total TEQ may alter thyroid function, but the study is limited by the small size and a short observation period (Pluim et al., 1992). Because the products of the thyroid control many aspects of metabolism, a better understanding of the relationship between 2,3,7,8-TCDD and thyroid function is important, especially regarding the developing human.

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8. DOSE-RESPONSE MODELING

8.1. INTRODUCTION

The current effort to reevaluate the risk of exposure to dioxins is being termed a *Biological Basis for Risk Assessment.* The underlying premise is that this may be a special case for a nonmutagenic, receptor-mediated carcinogen. The goal of this reassessment is to consider more mechanistically based models that are sufficiently credible to the scientific community.

Most of the information presented in the Introduction is found in more extensive detail in the other background chapters. It is useful to summarize the salient features of those papers that have an impact on the development of dose-response models so that readers of this chapter will be able to evaluate the scientific foundation on which the dose-response models presented in this chapter are based.

2,3,7,8-TCDD is the most potent form of a broad family of xenobiotics that bind to an intracellular protein known as the Ah receptor. Other members of this family include halogenated hydrocarbons such as the PCBs, naphthalenes, and dibenzofurans, as well as nonhalogenated species such as 3-methylcholanthrene and β-naphthaflavone. The biological properties of dioxins have been investigated extensively in over 5,000 publications and abstracts since the identification of TCDD as a chloracnegen (Kimming and Schultz, 1957). Much of the biological activity of TCDD appears to follow the rank order binding affinity of the congeners and analogs to the Ah receptor (AhR). This rank order holds for toxic responses such as acute toxicity and teratogenicity and for changes in concentration of several hepatic proteins including the induction of cytochromes P-450IA1 and IA2 and the modulation of the estrogen receptor and epidermal growth factor receptor (EGFR). The relationship between AhR binding and carcinogenicity of TCDD is less clear. However, TCDD is a carcinogen in several strains of laboratory mice and rats, and the tumor sites include liver, thyroid, and the respiratory tract, as well as others. There is considerable evidence that TCDD does not interact directly with the DNA to cause mutations. The study most utilized for the cancer risk assessment of TCDD is that of Kociba et al. (1978). These authors reported an increase in hepatocellular carcinomas and hepatomas, along with

decreases in several endocrine tumors in female rats. Male rats were remarkably less susceptible to the hepatocarcinogenic actions of TCDD. However, there is no striking sex specificity in the hepatocarcinogenic actions of TCDD in mice.

The overall hypothesis of TCDD action, put forth by several groups, is based on the transcription of specific genes exemplified by the cytochrome P-450IA1 gene. The biological basis for this approach is outlined in Chapter 2, Mechanism(s) of Action. Although substantial gaps in our knowledge remain, it is accepted by most researchers that most if not all cellular responses to TCDD require interaction between TCDD and the Ah receptor. The binding of TCDD to AhR is similar, although not necessarily identical, to the interaction of many steroid hormones with their intracellular receptors, as pointed out by Gustafsson in a series of articles drawing comparisons between the AhR and the glucocorticoid receptor (Poellinger et al., 1986, 1987; Cuthill et al., 1988). DeVito et al. (1991) have also drawn an analogy between the action of TCDD and estradiol at their respective receptor proteins.

The binding of TCDD to AhR is reversible. However, subsequent events seem to reduce the likelihood of dissociation of the ligand:receptor complex. One such event that has been recently studied is the association of the ligand:receptor complex with another macromolecule, the so-called ARNT (AhR nuclear transport) protein (Hoffman et al., 1991). There may be a family of ARNT proteins that differ by cell types, which could account, in part, for the diversity of actions of TCDD in different tissues. The association of ARNT with the ligand-bound receptor induces some physical changes in the complex, which tends to reduce dissociation of the ligand and favors the movement and/or retention of the complex into the nucleus. Overall, the relationship between TCDD concentration and nuclear AhR-TCDD concentration appears to be linear, indicating that, at low ligand concentrations, ARNT is not a rate-limiting factor. In the case of transcriptional activation of the CYP1A1 gene, the AhR-ARNT-TCDD complex (activated TCDD complex) associates with specific elements in the genome called the xenobiotic (or dioxin) responsive elements (XREs or DREs). The association of the activated TCDD complex with the DRE is also reversible (Gasiewicz et al., 1991), and there is recent evidence that at least two DREs need to be occupied to transcriptionally activate the CYP1A1 gene (Chapter 2, Mechanism(s) of Action). The structure and amino acid sequence of the AhR protein have been reported

(Burbach et al., 1992; Ema et al., 1992). Each AhR appears to bind one molecule of TCDD, and at low concentrations of ligand (i.e., when [ligand] < < [receptor]), the binding of TCDD to AhR is likely to be linearly related to [TCDD].

Much of the sequence of events is analogous to the steroid receptors and the respective genomic response elements. This similarity helps us in proposing biological models of TCDD action and risk assessment. The steroid hormones and their receptors belong to a multigene family that includes the thyroid hormone receptors, oncogene products, glucocorticoids, mineralcorticoids, vitamin D, retinoids, androgens, estrogens, and progestins (Chapter 2, Mechanism(s) of Action; Evans et al., 1988). Biologically, these are all multipotent agents that induce a range of cellular responses in different organs, many at extremely low concentrations. They share a nuclear location for the transduction of ligand:receptor action, and their common mechanism of action is the regulation of gene expression (Jensen, 1991). Within the family of known receptors from these agents, there is considerable sequence homology and a common basic structure, consisting of a ligand-binding domain and a DNA-binding domain. The biological activity of these receptors is varyingly regulated by metals and by phosphorylation state. Some--but not all--hormone receptors may interact with chaperone-type proteins, subsequent to ligand binding, which transduce conformational changes and other events critical to nuclear translocation and DNA binding. The ARNT protein functions in this fashion (Hoffman et al., 1991). Other receptors are associated with so-called heat shock proteins or proteins that must be shed to transform the liganded receptor into a DNA-binding form, and the DNA-binding domain of some receptors contains zinc finger loops, although this does not seem to be the case for the Ah receptor.

The steroid hormone receptors, sometimes as liganded dimers, move to the nucleus and regulate gene transcription through specific DNA sequences near the target genes. These events are complex because of interactions between the liganded receptor and nuclear proteins that function as transcription factors by binding to other DNA sites upstream of the hormone responsive elements and the regulated gene. These transcription factors may regulate the binding affinity of the steroid hormone receptor itself to DNA (Muller et al., 1991). Additional complexity is introduced by the interactions among steroid hormone

receptors, at the genetic level, and by the effects of hormone upon the number, conformation, and localization of receptors. Down- and up-regulation of receptor gene transcription and receptor synthesis may also be involved in cell-level modulation of steroid hormone action. As pointed out by Muller et al. (1991), every step in the signal transduction pathway of these hormones, from receptor gene transcription to ligand:receptor:DNA action, is likely to be regulated by both dependent and independent factors. Moreover, nongenomic effects of steroid hormones occur rapidly and independent of nuclear translocation of liganded hormone receptors or in the presence of protein synthesis inhibitors (Roth and Grunfield, 1985).

Attempts to model the steps involved in signal transduction have examined events step-by-step as well as the overall set of reactions from entrance of hormone into the cell to cellular response. Of interest in this report is the information that may be available concerning the overall dose-response relationship for steroid hormones. The highly complex cascade of biological events that intervenes from hormone entrance to cell response may modulate hormone action in the following ways: It may amplify cell response, in the way that second messengers for membrane-associated receptors (such as neurotransmitter receptors) appear to amplify molecular signaling; it may transduce response in a manner proportional to concentration of hormone (that is, linearly); or it may introduce dampening into the response network. Amplification of signal transduction implies that, at some stage in a multistep process, more than one event is triggered as a consequence of one preceding event. Dampening implies that, at some stage, more than one event must occur before the next event is triggered. Linear transduction implies that the relationship of event to event, for all steps, is invariant.

In considering these possible dose-response relationships, it is likely to be important to distinguish among endogenous and exogenous ligands for the same steroid hormone receptor, particularly if the two types of ligands differ in rates of turnover (degradability) or affinity for the receptor. We are hampered in our inferences for the dioxins because the endogenous ligand(s), if present, has not yet been identified, and thus we are not certain if TCDD is more or less stable than this ligand, if its affinity is higher or lower than an endogenous ligand, or if an endogenous ligand would act as an agonist or antagonist for

dioxin-like effects. With respect to stability, it is unlikely that an endogenous ligand would be as stable as TCDD. Most endogenous ligands for steroid hormone and other receptors are rapidly cleared, either by compartmentation (as with neurotransmitter reuptake processes) or by enzymatic degradation, as with steroids. With respect to kinetics of binding of TCDD, its in vivo affinity for the receptor is extremely high, in the 10^{-11} range. If the affinity for the natural ligand is even higher, then it is likely that the overall relationships between natural ligand and receptor are even stronger than those we may explicate for TCDD; if it is lower, then it would be an unusual member of the steroid hormone family. Of course, differences in affinity, if these exist, may not influence the overall kinetics of the dose-response relationship as much as differences in the number of events required to trigger the reaction from step to step.

Evaluation of dose-response relationships for receptor-mediated events requires information on the quantitative relationships between ligand concentration, receptor occupancy, and biological response. Roth and Grunfeld in *The Textbook of Endocrinology* (1985) state:

> At very low concentrations of hormone ([H] < < Kd), receptor occupancy occurs but may be trivial; i.e., the curve approaches 0% occupancy of receptors. But if there are 10,000 receptors per cell (a reasonable number for most systems), the absolute number of complexes formed is respectable even at low hormone concentrations. One advantage of this arrangement is that the system is more sensitive to changes in hormone concentration; at receptor occupancy (occupied receptors/total receptors, or [HR]/[Ro]), below 10%, [HR] is linearly related to [H], whereas at occupancies of 10 to 90%, [HR] is linear with log[H]--a given increase in [H] is more effective in generating HR at the lowest part of the curve than at the middle.

Figure 8-1 illustrates a situation where there is a proportional relationship between receptor occupancy and biological response. In this situation occupancy of one receptor would produce a response although it would be unlikely that this response could be detected. Moreover, the biological significance of such a response may be negligible, but this is not known and it may vary with end point as well as with developmental stage and cell type. It

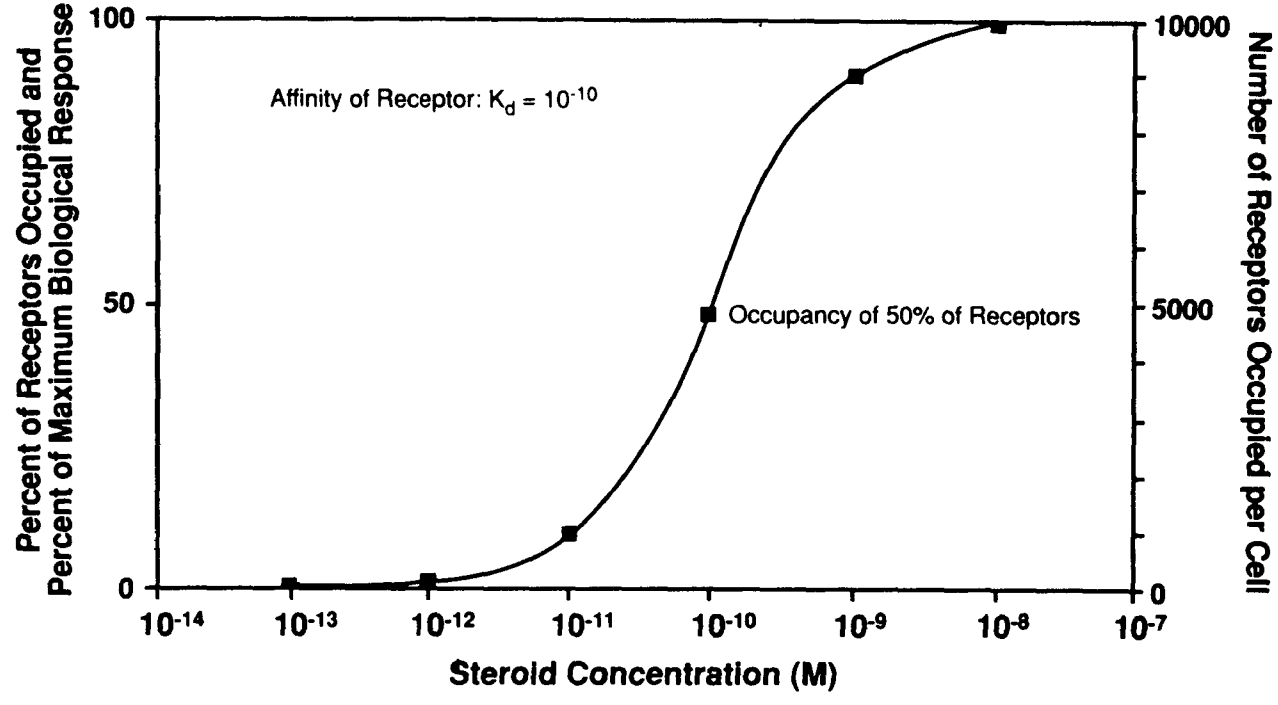

Figure 8-1. Dose/response graph showing proportional relationship between receptor occupancy and biological response (semilog scale)

Source: Modified from Roth and Grunfeld, 1985 (Figure 4-36)

is important to note that the data in Figure 8-1 are plotted on a semilog scale. If the same data are plotted arithmetically (Figure 8-2), then the shape of the dose-response curve readily conveys the linear relationship between receptor occupancy and biological response at lower concentrations and saturation at higher concentrations.

Such a simple proportional relationship does not explain the diversity of biological responses that can be elicited by a single hormone utilizing a single receptor. For example, low concentrations of insulin produce much greater effects on fat cells than on muscle cells. These differences are due to tissue- and cell-specific factors that modulate the qualitative relationship between receptor occupancy and response. Similarly, it is expected that there are markedly different dose-response relationships for different effects of TCDD. Coordinated biological responses, such as TCDD-mediated increases in cell proliferation, likely involve other hormone systems, which means that the dose-response relationships for relatively simple responses (i.e., CYP1A1 induction) may not accurately predict dose-response relationships for complex responses such as cancer. As we gain more understanding of the entire sequence of events responsible for TCDD-mediated toxic effects, we will enhance our ability to more accurately predict dose-response relationships. The mechanism(s) responsible for qualitative and quantitative diversity in receptor-mediated responses will be discussed in more detail in Section 8.7, Knowledge Gaps.

Cancer is a multistep, multistage disease in which several operationally defined events have been described primarily on the basis of assay systems developed to detect these events. These events are initiation, fixation, promotion, and progression, as shown in Figure 8-3. Although this schematic arrays these events in a linear progression, it is important to realize that there are multiple pathways by which a cell may progress through these stages from an early alteration in gene structure or expression to the expansion of a clone into a tumor. The early alteration in gene structure or expression is often referred to as initiation. Structural damage to DNA, through alkylation or deletion, is an example of initiation. This initial event is immortalized, or stabilized, in the daughter cells of the initiated cell through the processes of fixation. Promotion is the enhanced growth of the cell population with fixed genetic damage; promotion may be supported by hormones and other modifications in cell growth and proliferation. Progression is a term used to describe additional alterations in

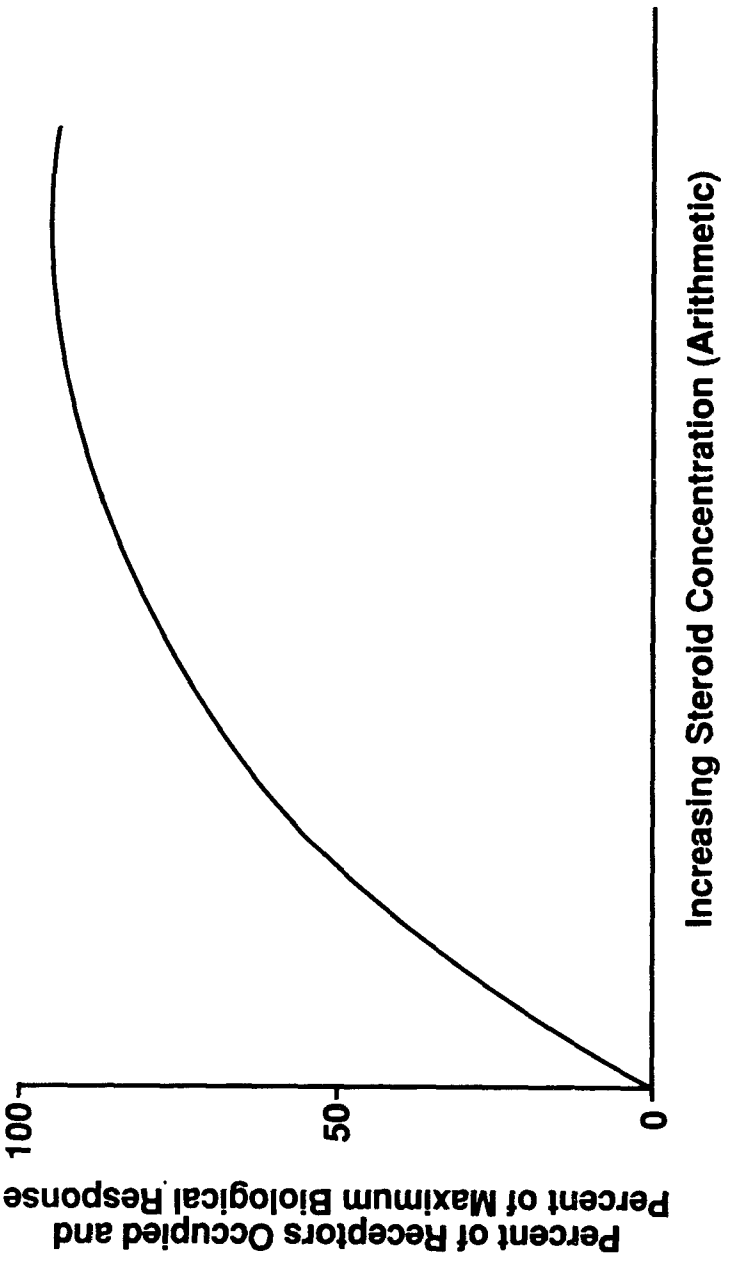

Figure 8-2. Dose/response graph showing proportional relationship between receptor occupancy and biological response (arithmetic scale)

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Figure 8-3. Multistage carcinogenesis

Source: Portier and Kopp-Schneider, 1991

gene structure or expression, such as second mutations for colon cancer, that appear to be necessary in the growth of the clone into a clinical end stage. These events are not necessarily ordered in this sequence, nor is it clear that distinctly different events--genotoxic and epigenetic--are involved in each stage. Some of these events are often defined by the test systems used to assay for their occurrence: For instance, initiation is often equated with mutation, such as the mutations that are detectable in in vitro bacterial assays of the Ames type. Promotion is often equated with a positive result in an experimental paradigm of sequential treatment of animals with a strong mutagen, followed by a putative "promoter."

Dioxin is an operational promoter, as defined in assay systems of skin and liver in mice and rats, respectively (Pitot et al., 1987; Clark et al., 1991; Lucier et al., 1991). Since many hormones are promoters (Pitot and Dragan, 1991; Lucier, 1992), it is not surprising that dioxin has these properties as well. The interactions of dioxin with hormones are complex (see below), and in cancer bioassays there is evidence for interactions among dioxin and sex hormones, in that the rates of liver tumors in rodents are much higher in females than in males (National Toxicology Program [NTP]), and ovariectomy suppresses dioxin promotion of diethylnitrosamine (DEN)-initiated liver tumors (Lucier et al., 1991). Dioxin is also a complete carcinogen; that is, it induces increased tumor yields in experimental animals not pretreated with strong mutagens (Kociba et al., 1978; NTP, 1982). Dioxin is not a mutagen in in vitro systems commonly used to detect mutation through DNA damage. There is some evidence for in vivo clastogenicity of DNA (increased chromosomal breaks) in animals exposed to dioxin (Stohs et al., 1990). These data have presented challenges to the application of general models for cancer risk assessment, which are based on assumptions of mutagenesis as a fundamental mechanism for chemical- or radiation-induced cancer.

The general approach of the U.S. EPA to regulation of carcinogens is to use the Armitage-Doll model of carcinogenesis (Figure 8-4). In this model, the movement of cells from one stage to the other is assumed to be due to a sequence of mutations similar to the step of initiation/fixation discussed above. As with any mathematical model, specific forms must be chosen for the rate constants that define the process. The EPA formulation of this model assumes the mutation rates [μ,(d,t)] are a linear function of dose and are constant over time. These assumptions result in a cumulative tumor incidence rate that can be

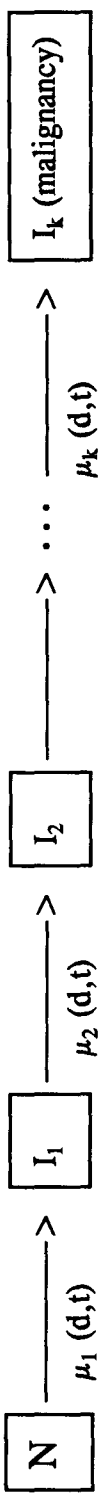


Figure 8-4. The Armitage-Doll model of carcinogenesis

approximated by a polynomial function of dose with coefficient q_i for dose^i, i=0,1,2,...k. In the low-dose region, the risk is dominated by the linear term in the polynomial (q_1). EPA generally uses a 95% upper-confidence limit (q_1 on the linear term of this formulation of the multistage model for cancer risk assessment. This model, using the 95% upper-confidence limit on the linear term, is referred to as the linearized multistage (LMS) model. However, this choice has not been predicated solely on the correctness of a K-mutation process of cancer development. The linearized mathematical properties of the multistage model can be appropriate for a larger class of mechanisms: Dose-response behavior, which is linear at low dose, may have upward curvature in the intermediate range and show a downward curvature or saturation of response at high dose. In particular, arguments that a compound's action is additive to background biological processes lead to a linear response at low dose under rather general conditions (Crump et al., 1976). Therefore, for practical modeling purposes, it is important to address whether biological knowledge about the action of a carcinogen can fit the general dose-response shape predicted by the linearized multistage model.

Our knowledge of dioxins may challenge some of the simplifying assumptions that have been the biological basis for cancer risk assessment models. While dioxins do not possess the properties of an operationally defined "genotoxin" (using in vitro tests of mutagenicity), dioxins clearly affect gene expression (see below and also Whitlock chapter). These gene-level effects appear to involve a transcription regulator, the so-called dioxin receptor, which when liganded to dioxin and translocated to the nucleus is associated with specific recognition elements upstream of target genes whose transcription may be up-regulated or down-regulated by the dioxin receptor. It is difficult to fit these events into our current conceptual model of chemical carcinogenesis.

For other toxicological end points such as terata, organ toxicity, acute toxicity, etc., a threshold has often been assumed primarily as a matter of policy. For these end points, safety factors or uncertainty factors have been used to estimate no-effect exposure levels. This threshold approach is used by the World Health Organization to set acceptable daily intakes (ADIs) for direct and indirect food additives. EPA now uses the term reference dose. In distinction, EPA policy assumes the dose-response curve for excess carcinogenic risk to be linear through dose zero. Several mechanisms could generally lead to this form of

response, including direct mutational activity of the chemical agent and/or additivity to background rate of tumor formation (Portier, 1987). Since TCDD does not bind covalently to DNA and must exert its effects through receptor action, this default position must be carefully reexamined.

8.1.1. Introduction to Modeling for TCDD

There are several models under consideration at the present time, ranging from very simple to complex. It is obvious that the biology governing the toxicity of TCDD, beyond a few initial critical events, is not straightforward. These critical events, the first of which is binding to the Ah receptor, are generally response-independent. The response-dependent events are species-, gender-, organ-, tissue-, and perhaps cell-specific. If the binding to the AhR is essential but not sufficient for effects to occur, then the dose-response curve for this event (as well as the rate equations) should be a better predictor of biological action than dose as long as the dose-response curves for these subsequent actions are functionally similar to the receptor binding curves. In general, the available data indicate receptor involvement is necessary for most if not all low-dose actions of TCDD. Since the AhR has been detected in virtually all cells but all cells do not respond to TCDD, there must be other factors that are necessary for TCDD action. The roles of these cell-specific factors must be elucidated before there is a complete understanding of TCDD action. However, a model may be developed for specific end points by using available data and reasonable assumptions.

Several important factors have been generally accepted. One, TCDD is a member of a class of xenobiotics (and probably natural products) that is nonmutagenic, binds to a cellular receptor, and alters cell growth and development. Two, a significant amount of information is available for estimating risks from exposure to this compound and the default position of directly applying the LMS model as a function of dose needs to be reevaluated. Three, the biology of receptor-mediated events should be included in any modeling exercise for TCDD. The goal of the modeling is to use as much data as possible to reduce these uncertainties and to identify the areas where data gaps exist.

One difficulty with a novel, albeit biologically based, approach is that it is replacing paradigms (safety factors and LMS models) upon which the U.S. Government's risk

assessments have been based. There is no a priori reason to believe that a model based on greater experimental evidence will be more or less conservative than the LMS model. However, basing the modeling on a mechanistic understanding of the biochemistry of TCDD-induced toxicity should increase our confidence in the resultant risk estimates. As previously stated by Greenlee and collaborators (1991):

> Neither the position taken by U.S. EPA or by Environment Canada (and several other countries such as Germany and the Netherlands) is based on any detailed mechanistic understanding of receptor-mediated interactions between dioxin and target tissues. Biologically-based strategies use knowledge of the mechanistic events in the various steps in the scheme for risk assessment. Interspecies extrapolation strategies would be conducted based on how these mechanistic steps vary from species to species. There are numerous steps that can be examined mechanistically, and fairly ambitious programs have been proposed to examine the mechanistic details of many or most of these individual steps. More focused risk assessment approaches are also being proposed based on examination of individual steps believed to be critical in establishing the overall shape of the dose-response curve for the induction of tumors (or other toxic end points) by dioxin.

This chapter examines several end points and focuses on dose-response models for cancer in laboratory animals and humans. It also evaluates the use of biomarkers of TCDD action as surrogates for modeling receptor-mediated events. In addition, the chapter presents a section titled Knowledge Gaps. Critical examination of this section can lead to new experiments that will add to our knowledge of TCDD action. Increased information on key molecular, cellular, and tissue-specific events will be important to validate a new risk paradigm for TCDD and perhaps other receptor-mediated nonmutagenic toxicants. This chapter focuses on cancer risk modeling. We review some critical data on noncancer end points, but we do not attempt to model them. These end points are clearly important when considering the public health risk of dioxins. However, the lack of knowledge on molecular action and molecular dosimetry limits our ability to propose mechanistically based mathematical models of noncancer end points at this time.

Mathematical modeling can be a powerful tool for understanding and combining information on complex biological phenomena. The development and use of mechanistically based mathematical models are illustrated by Figure 8-5. The beginning point is generally a series of experiments studying a xenobiotic agent. The experimental results (data) can indicate a mechanism, which leads to the creation of a mathematical model. The model is used to make inferences that are then validated against the existing knowledge base for the agent and effect under study. This can then lead to new experiments, which support further laps through the model development loop. On each pass through the loop, the model either gains additional validation by predicting the new experimental results or it is modified to encompass new results without sacrificing its base in previous results. In either case, subsequent loops through the model generally increase our confidence in accepting (or rejecting) a final model (although it may be difficult or impossible to quantify this confidence).

Confidence in any one model is not only dependent on the information available for that compound but also supported by the information available on other systems that act similarly and for which models have already been developed. In the case of TCDD, the modeling of effects will be greatly enhanced by existing information on the receptor-based systems and general work in physiologically based pharmacokinetic models and in tumor incidence modeling.

There is no one model development loop for any given compound or effect. Instead, there are unusually numerous pathways leading to the development of a mechanistic model. In modeling the effects of TCDD, there are many smaller model development circles that make up the larger overall model. For example, a mechanistic approach to TCDD-induced carcinogenicity must include models of exposure, tissue distribution, tissue diffusion, cellular biochemistry, cellular action, tumor incidence, and cancer mortality. At each stage and for each model, data must be collected and understood in order for the model to be valid and acceptable as a tool for understanding the observed effects and for predicting the effects of TCDD outside of the relatively limited range of experimental or epidemiological findings.

The use of mechanistically based modeling to extrapolate risks of exposure patterns and doses outside the range of the data is in its infancy. Even though there may be high

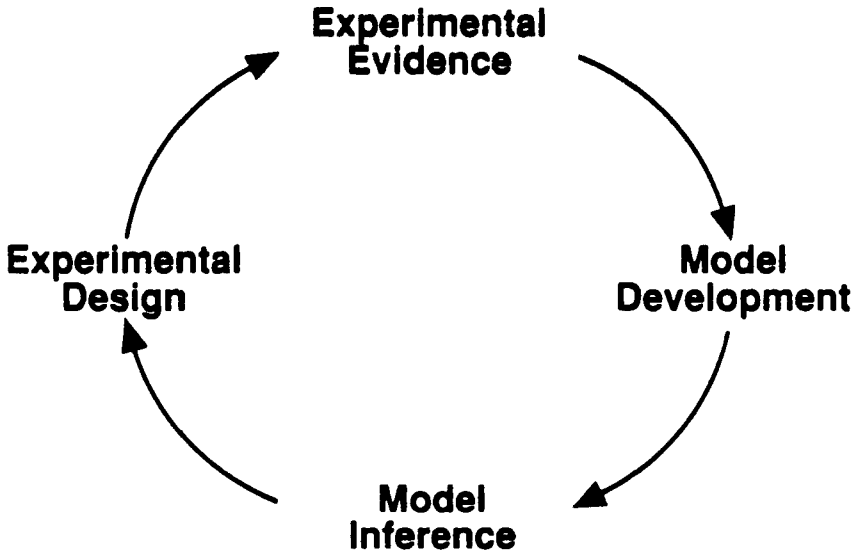


Figure 8-5. Developing a mechanistically based mathematical model

confidence in the ability of the model to predict experimental results, there could be low confidence in the ability of the model to predict outside the range of data. The use of models in risk assessment thus demands a careful scrutiny of the behavior of the model under a variety of exposure scenarios. This scrutiny has not generally been applied in science to the use of mechanistic models. It is important to note that mechanistic modeling has a role to aid in explaining and understanding experimental results, separate from its use in risk assessment. Our confidence in the methods used in mechanistic modeling will differ with use. For a historical perspective, it is important to recognize that this is not the first loop through the cycle of mechanistic modeling for carcinogenesis. Early risk assessments based on tolerance distributions used the then current understanding of carcinogenesis to develop statistical models that could be used for risk estimation (Mantel and Bryan, 1961). Later use of the LMS model was based on an understanding of carcinogenic mechanisms. Thus, this current exercise is a part of the process of mechanistically based modeling for risk assessment purposes.

In any realistic and practical modeling exercise, the major components of the model revolve around the estimation of model parameters utilizing statistical tools. These tools range from very simple techniques, such as estimating a mean, to extremely complicated approaches, such as estimation via maximizing a statistical likelihood. The estimation of parameters is not done in a vacuum but is tied to the data available to characterize the model. The way in which data are used to estimate those model parameters is the major component in determining the confidence placed in any mathematical model. Fundamentally, sufficient data need to be available to show that the model accurately represents at least what is known of biological processes that are associated with toxic events.

In modeling biological phenomena, the data can be divided into four broad categories, as shown in Table 8-1. At the top are effects observed in the whole animal. Examples of data in this category are survival of the organism, ability to reproduce, and overall function of the organism (e.g., behavioral data). Going from whole organism to tissue/organ system responses to cellular responses down to biochemical responses in the cell, the levels of data are increasingly specific and reductionist. The data range from the bottom of Table 8-1, which is extremely mechanistic and deals with the interactions between molecules, to the data

Table 8-1. Examples of Levels of Information Available for Estimating Parameters in Dose-Response Modeling

Organism	Morbidity
	Mortality
	Fertility
	Improper development/function
Tissue	Hyperplasia
	Hypertrophy
	Tumorigenesis
	Chemical distribution/disposition
Cell	Mitosis
	Cell death
	Cytoarchitectural pathology
Biochemical	Gene expression
	Protein levels
	Receptor binding
	Adduct formation

at the top of Table 8-1, which is often no more than counting bodies. All of this information is relevant and must be incorporated into a mathematical model aimed at understanding the specific biological response.

Mathematical models that incorporate parameters that are mechanistic in nature do not automatically constitute "mechanistic models." The types of data available for the model and the method by which these data are incorporated into the model determine if a model is truly "mechanistic," that is, soundly based on the biology rather than simply a curve fit to the same data.

There are two basic ways in which biological effects can be estimated. The first and most common approach is a "top-down" approach. In the top-down approach, data on the effect of interest (e.g., carcinogenicity) are modeled directly by applying statistical tools to link the observed data (e.g., tumor incidence data from a carcinogenicity experiment) to a model (e.g., the multistage model of carcinogenesis). This approach is extremely powerful in its ability to describe the observed results and to generate hypotheses about model parameters and the potential effects of changes in these parameters. Where this modeling approach begins to lack credibility is in its ability to predict responses outside the range of the data currently being evaluated. Even when the model being applied to the data is mechanistic in the sense that the model parameters are tied to some mechanism for the toxic effect (e.g., mutation rates and molecular effects), without direct evidence concerning the value for this parameter or even evidence supporting the particular structure of the model, one is basically left with a curve fit to the data. The historical application of the LMS model in risk assessment has been in this fashion.

True mechanistic modeling must be viewed in a different fashion. In this case, the model structure and the parameters in the model are derived in a "bottom-up" fashion. The mechanistic parameters in the model are estimated directly from mechanistic data rather than from effects data or data one level higher in the hierarchy of data illustrated in Table 8-1. The goal of true mechanistic modeling is to explain all or most known results relating to the process under study in a way that is reasonable in its biology and soundly rooted in the data at hand. In this case, biological confidence in predictions from the model would be much higher than that from the "curve fitting" approach.

In practice, it is generally impossible to completely eliminate curve fitting from mechanistic modeling. At some point in the modeling process, gaps must be filled to relate the mechanistic effects to the observed toxic effects. It is generally at this point that some amount of curve fitting is necessary. Although not technically mechanistic modeling, this combined approach is preferred to simple curve fitting when inferences outside of the range of the toxic effects data are desired.

This is not intended to infer that with mechanistic modeling we can get a precise estimate of risk of a toxic effect outside the range of the data or even necessarily a more precise estimate of risk than with curve fitting alone. Without data, the statistical issue of the accuracy of a prediction cannot be easily addressed. Thus, while there may be greater biological confidence in extrapolated results, it is unlikely that an increased statistical confidence can be demonstrated. However, for each level and type of data, there are ranges of exposure beyond which it is impossible to demonstrate an effect given the practical constraints on the experimental protocols. In general, effects can be demonstrated at lower exposures for data at the bottom of Table 8-1 compared to the data at the top. If this is the case, there may be both increased biological confidence in extrapolated results and increased statistical accuracy. This is also not intended to infer that models derived through curved fitting should always be given less weight. Many of the advances in biology and science are due to the application (either formally or informally) of a model to data at a higher level in order to generate hypotheses about effects at lower levels. The major difference between the application of a "curve-fit" model in basic biology and that in risk estimation is that in basic biology one is creating hypotheses which at some point can be tested. In risk estimation, it is unlikely that one will ever be able to validate extrapolated risk estimates.

Many side issues are also related to the use of this model development loop in trying to understand a biological mechanism. One of considerable importance is experimental design. For mechanistic modeling aimed at risk assessment, we are just beginning to understand the types of experiments that may assist us. In general design situations, one would have a mechanism in mind, qualitatively describe that mechanism and form the structure of a mechanistic model, and make educated guesses about the parameters of this model, then use the quantitative model to develop experimental designs that are optimally

relevant to characterizing the mechanism. For the purpose of risk estimation, this basic outline holds. There are also some simple design rules that are not required but would aid in the extrapolation of results to doses outside the observed response range and to humans from animals. A few of these design points are listed in Table 8-2.

TCDD can be considered as a prototype for exploring and examining the ability of mechanistic modeling to improve the accuracy of quantitative risk assessment. The database for a mechanistic modeling approach to TCDD is very extensive and contains a considerable amount of information on low-dose behavior. In addition, there is some concordance between human data and experimental evidence in animals. On the other hand, some aspects of the mechanism by which TCDD induces its effects, such as binding to the Ah receptor, have not been modeled extensively, and thus we are in only the first few loops through the model development cycle shown in Figure 8-5. Because of this, several competing mechanistic theories may agree with the existing data, adding to the uncertainty in any projected risk estimates. This outcome is inevitable for the application of the technology of mechanistic modeling to a new area. To reiterate an earlier point, mechanistic modeling can aid in explaining and understanding experimental results, beyond its proposed use in risk assessment. Our confidence in the methods used in mechanistic modeling will differ depending on its use. As we know more about the limitations of current data and current methods for the application of mechanistic models to risk estimation, we can improve experimental designs and significantly improve the process. Since this is an early attempt along these lines, we must be cautious in coming to a judgment concerning the overall utility of mechanistic modeling as an important tool for risk assessment.

In the National Academy of Sciences report *Risk Assessment in the Federal Government: Managing the Process* (National Research Council, 1983), "dose-response assessment" referred to the process of estimating the expected incidence of response for various exposure levels in animals and humans. Tissue response is not always directly related to exposure. This can be due to saturation and activation of metabolic pathways (Hoel et al., 1983); influence of competing pathways having different efficiencies of action for the parent compound and/or its key metabolites; and factors such as cytotoxicity, mitogenesis, or endocrine influences that can radically modify the homeostatic state of the

Table 8-2. Design Considerations for Risk Assessment Purposes

Aspects of risk estimation	Design points to consider
Mechanism	Multiple times of observation
	Multiple doses
	Multiple ages at exposure
	Pharmacokinetics
Species extrapolation	Multiple species
	Both sexes
	Tissue concentrations (including blood)
	System clearance
	Essential interactions with endocrine systems
Other	Multiple end points for individual animals

tissue. These complex interactions can result in markedly nonlinear dose response; nonlinearities could lead to risk estimates that may be greater or less than the risk derived from a linear model. Because of the potential for nonlinearities, it is essential to distinguish between exposure level and dose to critical tissue or cell when modeling risks from exposure to xenobiotics. It is also essential that we understand the quantitative relationship between target tissue dose and changes in gene expression. This is especially true when extrapolating to low doses and extrapolating across species.

For dioxin, the abundance of data on many levels allows one to create a collection of models that include the determination of the quantitative relationship between dioxin exposure and tissue concentration, tissue concentration and cellular action, cellular action and tissue response, and finally tissue response and host survival (Portier et al., 1984). This portion of the reevaluation of dioxin risks entails the description and development of mechanistically based mathematical models of the effects of TCDD. This includes a discussion of the extrapolation of tissue dosimetry and response from high-dose exposures to those expected at much lower exposure based on empirical relationships used to derive explicit, though incomplete, biologically based mechanistic models of the events involved in the toxic action of dioxin.

Biological modeling is the process of developing mathematical descriptions of the interrelationships among the mechanistic determinants of those toxic events resulting from exposure to TCDD. Research with dioxin has focused on biological responses at the levels of organization shown in Table 8-1 (i.e., biochemical, cellular, tissue, and organism). At each level of organization, we focus on the mechanisms responsible for these observations. Mechanism refers to the critical biological factors that regulate occurrence, incidence, and severity of a particular factor and the nature of the interrelationships among these factors. The details of the mechanisms of interaction differ markedly for the various levels of biological organization with specific determinants of the behavior at each level driving the creation on an appropriate quantitative model.

For dioxin, the mechanisms of three processes are of primary interest: (1) the dosimetry of dioxin throughout the body and specifically to target tissues; (2) the molecular interactions between dioxin and tissues, emphasizing the activation of gene transcription and

increases in cellular protein concentrations of specific growth-regulatory gene products and specific cytochromes; and (3) the progressive tissue-level alterations resulting from these interactions that lead, eventually, to toxicity. The modeling process involves identification of the mechanistic determination of the dose-response continuum through experimentation and the encoding of these processes in mathematical equations. The extent to which model predictions coincide with experimental results is a test of the validity of the model structure. After validation, the model can be used for risk assessment. In addition to their use in risk assessment, these models have importance for aiding in the design of future research, both in terms of a basic understanding of dioxin toxicity and further risk estimation.

The following sections discuss the mechanistic biological modeling for dioxin with regard to dosimetry, induction of gene transcription, and tissue response, especially those associated with hepatic carcinogenesis. This modeling effort follows a natural progression related to the kind of information available at the time at which the model was developed. We will begin with a review of tissue concentration followed by modulation of protein concentrations and tissue response.

8.1.2. Dose Delivery, Tissue Modeling, and Biochemical Modeling (see Appendices A and B for complete manuscripts by Kohn et al., 1993 and Andersen et al., 1993b)

Tissue dosimetry encompasses the absorption, distribution, metabolism, and elimination of dioxin from tissues within the body. The determinants of dioxin dosimetry in the body include physicochemical properties (e.g., diffusion constants, partitioning constants, kinetic constants, and biochemical parameters for metabolism) and physiological parameters (e.g., organ flows and volumes). The mathematical structure that describes the interconnections among these determinants constitutes a mathematical model for the tissue dosimetry of dioxin. The model used in the context of this reassessment is a physiologically based pharmacokinetic (PB-PK) model. These types of models describe the pharmacokinetics of dioxin with a series of mass-balance differential equations. These models have been validated in the observable response range for numerous compounds in both animals and humans, making them extremely useful for risk assessment, especially for cross-species extrapolation. In addition, they aid in extrapolation from one chemical to other structurally

related chemicals since many of the components of the model can be deduced for structurally related compounds. The development of PB-PK models is discussed for general use (Gerlowski and Jain, 1983) and for use in risk assessment by Clewell and Andersen (1985).

In brief, a PB-PK model consists of a series of compartments that are based on the anatomy and physiology of the test animals; hence, the term PB-PK. The time course of behavior in each compartment is defined by an equation containing terms for input and loss of chemical. For example, if C_ℓ represents the concentration of compound in a tissue (ℓ) and C_B the concentration of compound in blood (B), one of the simplest relationships one might use is:

$$\frac{\partial C_\ell}{\partial t} = r_{B\ell}\, C_B - r_{\ell B}\, C_\ell - r_m\, C_\ell \qquad [1]$$

where $\partial C_\ell/\partial t$ represents the change in the concentration in the tissue over time (t), $r_{B\ell}$ is the rate (per unit concentration) of the movement of the compound from blood to tissue, $r_{\ell B}$ the rate from tissue to blood, and r_m the rate of metabolism in the tissue. Equations of this form will be used in mass balance modeling of the pharmacokinetic processing of TCDD.

Several PB-PK models have been developed for dioxin and related chemicals (see Chapter 1, Disposition and Pharmacokinetics, for a brief overview). PCBs have been extensively studied (Lutz et al., 1977, 1984; Matthews and Dedrick, 1984). King et al. (1983) modeled the kinetics of 2,3 7,8-TCDF in several species, and Kissel and Robarge (1988) proposed a human PB-PK model.

The development of PB-PK models for TCDD began with work by Leung et al. (1988) in mice. This model was extended to Sprague-Dawley rats by Leung et al. (1990a) and to 2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin in mice (Leung et al., 1990b). Since many of the regulatory standards for dioxin have been based on a finding of hepatocarcinogenicity in female Sprague-Dawley rats, we will focus on the model by Leung et al. (1990a) in this strain and species.

The Leung et al. (1990a) PB-PK model contains five tissue compartments including blood, liver, fat, slowly perfused tissue, and richly perfused tissue. This early model is

blood flow limited, a condition that is appropriate when membrane diffusion is much more rapid than blood flow to the tissue. Thus, in this PB-PK model, the tissue/tissue blood compartments are lumped together as a single compartment in which the effluent venous blood concentration of TCDD is equilibrated with the tissue concentration. The model includes a TCDD-binding component in blood described by a linear process with an effective equilibrium between the bound and free TCDD given by a binding constant, K_{AB}. It also includes binding of TCDD to two classes of protein in the liver: one corresponding to the high-affinity, low-capacity Ah receptor and the other to a lower affinity, higher capacity microsomal protein (CYP1A2), which is inducible by TCDD. In the formulation of this PB-PK model by Leung et al. (1990a), both types of binding proteins are explicitly defined using an instantaneous correspondence between occupancy and induction using separate binding capacities and dissociation constants for each protein. These binding reactions are modeled via Michaelis-Menten equations.

In the Leung et al. (1990a) model, the tissue storage capacity depends on the partition coefficients (assumed to be linear with concentration) and the specific protein binding. Dioxin is very lipophilic and is found in higher concentrations in liver than would be expected based on partition coefficients. The incorporation of a term for specific binding of dioxin to a liver protein used by Leung et al. (1988) is a modification over earlier models for these lipophilic compounds.

In various studies, dioxin has been administered by intravenous administration, intraperitoneal injection, oral feeding or intubation (gavage), or subcutaneous (sc) injection. In the PB-PK modeling framework, intravenous injection can be described by starting the integration with an initial mass equal to the dose in the blood compartment. Oral intubation and sc injection can be modeled as if they adhere to first-order uptake kinetics with dioxin appearing in the liver blood after oral administration and in the mixed venous blood after sc injection. Feeding was modeled by Leung et al. (1988, 1990a) as a zero-order input on days that dioxin was included in the diet. These descriptions of the routes of uptake are clearly

not defined in specific physiological terms; they are empirical attempts to estimate an overall rate of uptake of TCDD into the PB-PK model. This is one area in which additional research could improve dose-response modeling for TCDD. Efforts to provide more biological details concerning the physiological basis of absorption across these various membranes, including intact skin, would prove valuable for exposure assessments with dioxin. With the iodinated analog, 2-iodo,3,7,8-trichlorodibenzo-*p*-dioxin, the estimated rate constant for oral absorption was considerably larger in induced (0.15/hour) than in naive animals (0.4/hour). The physiological basis of this change is unknown.

With many volatile organic chemicals there are convenient in vitro methods for estimating partition coefficients (Gargas et al., 1989). For TCDD and other highly lipophilic, essentially nonvolatile compounds, there are no reliable in vitro methods and these constants have to be estimated from measurements of tissue and blood concentrations in exposed animals. This leads to a difficulty in differentiating between specific-tissue binding and the partitioning to the tissue. Leung et al. (1990a) overcame this problem by assuming binding occurred only in the liver and that the liver partition coefficient was the same as the kidney. This permitted estimation of the relative binding capacities and affinities of specific hepatic proteins. The predictions from this modeling exercise prompted a series of experiments to examine the nature of these binding proteins in mice (Poland et al., 1989a, b).

Metabolic clearance was modeled as a first-order process. In the mouse with the iodo-derivative, dioxin pretreatment at maximally inducible levels caused a threefold increase in the rate of metabolism. There is no evidence to suggest an increase of metabolism in the rat for TCDD. Chapter 1, Disposition and Pharmacokinetics, discusses pathways for TCDD metabolism.

Finally, Leung et al. (1990a) kept all physiological parameters (e.g., flow rates, tissue weights) constant over the lifetime of the animal.

Dioxin and dioxin analogs have dose- and time-dependent kinetics in both rodents (Kociba et al., 1976; Poland et al., 1989a; Abraham et al., 1988; Rose et al., 1976; Tritscher et al., 1992) and humans (Pirkle et al., 1989; Carrier, 1991). For single- and short-duration exposures, as the exposure level increases, the proportion of total dose found

in the liver increases. For chronic exposures, there appears to be a linear relationship between dose and tissue concentration in the gavage study of Tritscher et al. (1992), but this may be simply an inability to observe nonlinearity in liver in intermediate dose ranges. The Leung et al. (1990a) model adequately predicts the tissue concentrations observed by Rose et al. (1976) but did considerably worse at predicting the results observed by Kociba et al. (1976), underpredicting concentrations at the lowest dose by a factor of 3.2 and overpredicting concentrations at the highest dose by a factor of 2. The data of Abraham et al. (1988) and Tritscher et al. (1992) were not available at the time this model was developed, but at least for the data of Tritscher et al. (1992), this model has been shown to overpredict the tissue concentrations (Kohn et al., 1993).

As mentioned earlier, the default position of the EPA in estimating risks from exposure to xenobiotics involves the use of a model that produces risk that is proportionate to dose for low doses (low-dose linearity). Thus, in discussing the models and submodels that form a basis for a mechanistic model for TCDD, we will focus on aspects of the model that could lead to nonproportional response for low environmental doses. The model of Leung et al. (1990a) predicts slight nonlinearity between administered dose and tissue concentration in the experimental dose range. In the low-dose range, the model predicts a linear relationship between dose and concentration. (They argue, however, that tissue dose alone should not be used for risk assessment for TCDD due to the large species specificity in the ability of TCDD to elicit toxicity). They instead suggest that use of time-weighted receptor occupancy linked with a two-stage model of carcinogenesis as a better approach to risk estimation. The time-weighted receptor occupancy predictions derived from the Leung et al. (1990a) model are linear in the low-dose region, reaching saturation in the range of high doses used to assess the toxicity of TCDD. Comparison of this proposed effective dose with the two-stage model of carcinogenesis is presented later in this chapter.

Looking at one aspect of modeling TCDD's effects, Portier et al. (1993) examined the relationship between tissue concentration and the modulation of the three liver proteins by TCDD in intact female Sprague-Dawley rats. The proteins studied included the induction of two hepatic cytochrome P-450 isozymes, CYP1A1 and CYP1A2, and the reduction in maximal binding to the EGF receptor in the hepatic plasma membrane. The modulation of

these proteins is believed to be mediated through TCDD binding to the Ah receptor. Then, as described in earlier chapters, through a series of alterations in the receptor-dioxin complex, transport to the nucleus, binding to transcriptionally active recognition sites on DNA, activation of gene transcription, and alterations in gene MRNA products, CYP1A1 and CYP1A2 are induced. Reduction in maximal binding to the EGF receptor requires additional protein interactions.

General empirical models have been developed for the regulation of gene expression (Hargrove et al., 1990). This modeling approach includes mRNA production by a zero-order process and first-order degradation. Activation alters one or both of these rates. The production of protein is assumed to be directly related to mRNA concentration. A more specific pharmacodynamic model has been described to account for the induction of tyrosine aminotransferase (TAT) activity by the corticosteroid prednisolone (Nichols et al., 1989). In this induction model, the input prednisolone concentration is specified by the measured time course of prednisolone in plasma. Prednisolone binding to receptor is specified by association and dissociation rate constants. The prednisolone receptor binds DNA with a specified association rate constant, and the bound receptor recycles to cytosol with a transport time, T (effective compartment transport times are included to account for delays between interaction with DNA and the appearance of TAT activity). A power function can describe a nonlinear relationship between the concentration of prednisolone receptor and the production rate of protein. The actions of prednisolone and maintenance of its tissue concentration are much more short-lived than those of dioxin, and the modeling period of interest is only on the order of several hours to a day instead of days, weeks, or months as with dioxin.

The important relationships presented here are the association of dioxin with the Ah receptor and the association of the dioxin receptor complex with DNA. As described above, Leung et al. (1988) modeled the induction of CYP1A2 as being due to a basal amount of protein plus an additional amount of protein resulting from binding of TCDD to the Ah receptor. The extent of induction was calculated as instantaneously related to percent occupancy of the Ah receptor via a Michaelis-Menten type relationship. Changes in CYP1A1 and EGF receptor binding were not modeled by Leung et al. (1990a).

Portier et al. (1993) modeled the rate-limiting step in the induction of CYP1A1 and CYP1A2 following exposure to TCDD using a Hill equation. Hill equations are commonly used for modeling ligand-receptor binding data. This equation allows for both linear and nonlinear response below the maximal induction range. A complete discussion of Hill kinetics and other models for ligand-receptor binding is given by Boeynaems and Dumont (1980); examples of the use of Hill kinetics for ligand-receptor binding include the muscarinic acetylcholine receptors (Hulme et al., 1981), nicotinic acetylcholine receptors, opiate receptors (Blume, 1981), the Ah receptor (Gasiewicz, 1984), estrogen receptors (Notides et al., 1985), and glucocorticoid receptor (Sunahara et al., 1989). As a direct comparison to what was done by Leung et al. (1990a), it is interesting to note that the Hill model can be thought of as a very general kinetic model that includes standard Michaelis-Menten kinetics when the Hill exponent is 1. Portier et al. (1993) modeled the reduction in maximal binding to the EGF receptor also following Hill kinetics, but with TCDD reducing the binding from the maximum level from control valves. For all three proteins, proteolysis was assumed to follow Michaelis-Menten kinetics. The proposed models fit the data in the observable response range.

The major purpose of the paper by Portier et al. (1993) was to emphasize the importance of endogenous protein expression on the shape of the tissue concentration/ response curve. For each protein, they considered two separate models. In the first, the additional expression of protein induced by TCDD is independent of the basal level expression. Such a mechanism is similar to that used by Leung et al. Under this model protein expression is given by the equation:

$$\frac{\partial P}{\partial t} = B_p + \frac{V_m C^n}{K_d + C^n} - \frac{V_p P}{K_p + P} \qquad [2]$$

where P is the concentration of protein in the liver, B_p is the basal rate of production of protein, V_m is the maximal level of induction of protein by TCDD, K_d is the apparent dissociation constant for binding (in the rate-limiting step), C is the concentration of TCDD in the tissue, V_p is the maximal rate of proteolysis, K_p is the proteolysis rate constant, and n

is the Hill exponent. When the Hill exponent is estimated to be an integer, the estimate of n can be interpreted as corresponding to the effective number of binding sites that must be occupied for the effect of the binding reaction to be expressed. When the Hill exponent is not an integer, other molecular meanings apply. These are discussed in detail in Boeynaems and Dumont (1980).

The second model Portier et al. considered was one in which the basal expression of these proteins was due to a ligand that competed with TCDD for binding sites. This leads to equations of the form:

$$\frac{\partial P}{\partial t} = \frac{V_m (C+E)^n}{K_d + (C+E)^n} - \frac{V_p P}{K_p + P} \qquad [3]$$

where E refers to the concentration of this ligand in units of TCDD binding-affinity equivalents. Under steady-state conditions, equations (2) and (3) are simplified (Portier et al., 1993).

Using these simpler formulas, they see virtually no difference between the independent and additive models in the observable response range, even to the point of getting almost equal Hill coefficients in the two models for all three proteins. In the low-dose range where risk extrapolation would occur, the models differed depending on the value of the Hill coefficient.

In all cases, the additive model resulted in low-dose linearity. This is expected, since, under the additive model, each additional molecule of TCDD adds more ligand to the pool available for binding and thus increases the concentration of protein. Similar observations have been made with regard to statistical (Hoel, 1980) and mechanistic (Portier, 1987) models for tumor incidence. For CYP1A1, the Hill exponent was estimated to be ~ 2. When the Hill exponent is > 1, the independent model yields a nonlinear dose-response that is concave (threshold looking). For CYP1A2, the Hill exponent was estimated to be ~ 0.5. When the Hill exponent is estimated to be < 1, dose-response is again nonlinear, but in this case it is convex, indicating greater than linear increases in response for low doses. Finally,

for the EGF receptor, the Hill exponent was approximately 1, in which case the two models are identical.

Thus, even though these two basic models show almost identical response in the observable response region, their low-dose behavior is remarkably different. If either CYP1A1 or CYP1A2 levels had been used as dose surrogates for low-dose risk estimation, the choice of the independent or additive model would make a difference of several orders of magnitude in the risk estimates for humans. Using CYP1A1 as a dose surrogate, the independent model would predict much lower risk estimates than the additive model. For CYP1A2, the opposite occurs. For EGF receptor, there would be no difference.

Andersen et al. (1993b) modified the model of Leung et al. (1990a) to include Hill kinetics in the induction of CYP1A1 and CYP1A2 and to use a diffusion limited approach to the development of a PB-PK model as compared to the blood flow limited approach used by Leung et al. Diffusion limited modeling is preferred when diffusion into a tissue is less rapid than blood flow to a tissue. In the model used by Andersen et al. (1993b), each tissue has two subcompartments, the tissue blood compartment and the tissue itself. Free TCDD flows into the tissue blood compartment and, from there, diffuses into the tissue. There is no direct relationship between effluent venous concentrations and tissue concentration in this diffusion limited model. For TCDD, the diffusion limited approach is preferred due to the compound's potentially slow diffusion into the liver from blood (Kohn et al., 1993).

Binding of TCDD to the Ah receptor was modeled in a fashion identical to that used by Leung et al. (1990a). The concentration of CYP1A2 was modeled as before using a steady-state model, in this case, with Hill kinetics instead of a Michaelis-Menten model. The resulting equation is identical to that used by Portier et al. (1993) for the independent induction of CYP1A2 except that they related this to the concentration of Ah receptor/TCDD complex rather than the concentration of TCDD in the liver. Since they assume binding of TCDD to the Ah receptor follows Hill kinetics with a Hill coefficient of 1 (Michaelis-Menten kinetics), the model of Andersen et al. (1993b) approaches the independent induction model of Portier et al. (1993) for low doses.

The induction of CYP1A1 was modeled as a time-dependent process as in equation (2), again utilizing TCDD bound to the Ah receptor rather than tissue concentration of

TCDD. Most of the physiological constants and many of the pharmacological and biochemical constants used in the Leung et al. (1990a) model were changed for the Andersen et al. (1993b) model to correspond to Wistar rats. The parameters in the model were optimized to reproduce tissue distribution and CYP1A1-dependent enzyme activity in a study by Abraham et al. (1988) and liver and fat concentrations in a study by Krowke et al. (1989). For the longer exposure regimens and observation periods, changes in total body weight and the proportion of weight as fat compartment volume were included via piecewise constant values (changes occurred at 840 hours and 1,340 hours).

Andersen et al. (1993b) noted that the liver/fat concentration ratio changes as dose changes due to an increase in the amount of binding protein in the liver. For high doses in chronic exposure studies, this introduces a nonlinearity into the concentration of TCDD in the liver. In the low-dose region, because the Hill coefficient for CYP1A2 and for the Ah receptor are equal to 1, the liver concentration as a function of dose is still effectively linear (i.e., small doses of TCDD will bind to the Ah receptor increasing the amount of Ah receptor/TCDD complex, which then induces additional production of CYP1A2, which can bind to free dioxin). In the observable response range, there is a slight nonlinearity in the concentration of TCDD in the liver as a function of dose under chronic exposure (Andersen et al., 1992). This nonlinearity in the dose region of 1 to 100 ng/kg/day does not agree with the findings of Kociba et al. (1976) and Tritscher et al. (1992) for chronic exposure in Sprague-Dawley rats. The plateau in total liver concentration predicted by the model of Andersen et al. (1993b) does occur in the data of Kociba et al. (1976) and Tritscher et al. (1992), in the range of 100 ng/kg/day consistent with the 87 ng/kg/day predicted by Andersen et al. (1993b). However, the changes in liver/fat ratio observed by Andersen et al. (1993b) and supported by human evidence (Carrier, 1991) are a necessary part of the modeling for TCDD.

Finally, with regard to risk estimation, Andersen et al. (1993a) compared the induction of CYP1A1 and CYP1A2, the concentration of free TCDD in the liver, and the total concentration of TCDD in the liver to tumor incidence (Kociba et al., 1976) and the volume of altered hepatic foci (Pitot et al., 1987). Using a biweekly dosing regimen, TCDD was injected intramuscularly in female rats for 6 months. The concentration of TCDD in the

liver and the concentration of induced protein were integrated over time to get summary measures of internal exposure. They concluded that tumor promotion correlated more closely with predicted induction of CYP1A1 than the other integrated quantities. No formal measure was used to support this observation. Thus, without a formal measure of agreement, it is difficult to know if CYP1A2 induction can be rejected as correlating with tumor response or focal lesion volume. This is discussed later in this chapter. Finally, the choice of an independent induction model for CYP1A1 and a Hill coefficient >1 leads to nonlinear low-dose behavior. If the promotional effects of TCDD follow a similar mechanism, the risk from exposure at low doses will be negligible. For risk assessment, it is important to know if an additive model also fits these data and agrees with the promotional effects of TCDD since such a model will have different low-dose behavior than the independent model.

Kohn et al. (1993) expanded upon the model of Leung et al. (1990a) to include Hill kinetics, a restricted flow-limited PB-PK formulation, and an extensive model of the biochemistry of TCDD in the liver. The goal of the model was to explain TCDD-mediated alterations in hepatic proteins in the rat, specifically considering CYP1A1, CYP1A2, Ah receptor, EGF receptor, and estrogen receptor over a wide dose range. In addition, the model describes the distribution of TCDD to the various tissues, accounting for both time and dose effects observed by other researchers. The PB-PK models developed by Leung et al. (1990a) and Andersen et al. (1993b) relied on several single-dose data sets (Rose et al., 1976; Abraham et al., 1988) and were validated against dosimetry results from longer term subchronic and chronic dosing regimens (Kociba et al., 1976, 1978; Krowke et al., 1989). These and more recent studies in which female Sprague-Dawley rats received TCDD (Tritscher et al., 1992; Sewall et al., 1993) were used by Kohn et al. (1992) to model the pharmacokinetics and induction of gene products in this sex and species. Among the data reported by Tritscher et al. (1992) and Sewall et al. (1993) were (1) concentrations of TCDD in blood and liver and (2) concentrations of hepatic CYP1A1 and CYP1A2 and EGF receptor in the hepatocyte plasma membrane. Kohn et al. (1993) refer to their model as the NIEHS model. The tissue dosimetry for the NIEHS model was validated against the single dose and

chronic dosing regimen experiments employed by Leung et al. (1990a) and Andersen et al. (1993b).

In the biochemical effects portion of the NIEHS model, the binding of TCDD to the Ah receptor is modeled using explicit rate constants instead of dissociation equilibrium constants (equation 2 with $B_p = 0$). However, larger dissociation rates (k_d, k_i) were used leading to a formulation of the amount of TCDD-Ah-receptor complex similar to that used by Leung et al. (1990a) and Andersen et al. (1993b) Many of the other binding reactions in the model were handled similarly (e.g., TCDD binding to CYP1A2 and TCDD bound to blood). This is simply a numerical trick to avoid the necessity of solving for the concentration of TCDD in the liver using the mass conservation relationship described in Leung et al. (1990a).

The physiology described in the NIEHS model is dependent on the body weight of the animal. Body weight changes as a function of dose and age were recorded by Tritscher et al. (1992) and directly incorporated into the model via a smooth function. Tissue volumes and flows were calculated as an allometric formula based on recent work by Delp et al. (1991). To allow the model to fit the data of both Rose et al. (1976) and Tritscher et al. (1992), the NIEHS model includes loss of TCDD from the liver by lysis of dead cells where the rate of cell death (and the resulting lysis) was assumed to increase as a hyperbolic function of the cumulative exposure in the liver to unbound TCDD. No information regarding the rate of TCDD from lysed cells is available; therefore, this feature of the NIEHS model predicts a net contribution of TCDD clearance by TCDD-induced cell death.

In the biochemical effects portion of the NIEHS model, the Ah-receptor/TCDD complex up-regulates four proteins; CYP1A1, CYP1A2, the Ah receptor, and transforming growth factor-α (see Figure 1 in Appendix A). For all four proteins, synthesis and degradation rates are defined explicitly. Changes in CYP1A1, CYP1A2, and the Ah receptor are compared to data on these concentrations. The induction of EGF-like peptides is deduced from observations on human keratinocytes (Choi et al., 1991; Gaido et al., 1992) and is quantified based on an assumed interaction with the EGF receptor. However, TCDD-mediated induction of TGF-α has not been clearly demonstrated in liver (see Appendix A). Constitutive rates of expression for CYP1A2, Ah receptor, and EGF receptor are assumed

independent (equation 2) at the induced expression. This has no effect on low-dose rate extrapolation since the Hill coefficients for the induction of these proteins by the Ah receptor/TCDD complex were estimated to be 1. Induction of CYP1A1 was assumed to be based on additive induction (equation 3), but again the Hill exponent was estimated to be 1, leading to low-dose linearity under either model equation (2 or 3). Thus, the NIEHS model found that the induction of all gene products appears to be a hyperbolic function of dose without any apparent cooperativity (i.e., the value of the Hill exponent, n, in equation 2, is estimated to be 1). The discrepancy in the estimates of the Hill exponents between this model and the other models discussed (Portier et al., 1993; Andersen et al., 1993b; Kedderis et al., 1992) is probably related to the inclusion of induction of the Ah receptor in the NIEHS model.

In the NIEHS model, the Ah-receptor/TCDD complex down-regulates the estrogen receptor. It is assumed that the estrogen receptor-estrogen complex synergistically reacts with the Ah receptor/TCDD complex to transcriptionally activate gene(s) that regulate synthesis of an EGF-like peptide. This term was introduced to partially account for the observation of reduced TCDD tumor-promoting potency in ovariectomized females as compared to intact female rats (Lucier et al., 1991). This mechanism, although supported by some data (Clark et al., 1991; Sunahara et al., 1989), is speculative (Kohn et al., 1993) (see Appendices A and B).

There are basically three levels of complexity of PB-PK models for the effects of TCDD. First is the traditional PB-PK model by Leung et al. (1988) with the added complexity of protein binding in the liver. The next level of complexity is the model by Andersen et al. (1993b) using diffusion limited modeling and more detailed modulation of liver proteins. Finally, there is the model of Kohn et al. (1993) with extensive liver biochemistry. All three models have biological structure and encode hypotheses about the modulation of liver proteins by TCDD. However, for gene expression, all three models fall in between curve fitting and mechanistic modeling. In their derivation, the parameters were estimated using dose-time-response data for protein concentrations and enzyme activity, which are a direct consequence of gene expression. This constitutes curve fitting at this level. However, the structure of the models is derived from qualitative information on the

effects of TCDD; the PB-PK model, and even the biochemical model of Kohn et al. (1993), predict protein concentrations using data sets that were not included in the original derivation of the model and that were derived from designs other than those used to characterize the original model. This constitutes a mechanistic validation of the original models and places these exercises in the realm of partial curve fitting and partial mechanistic modeling.

In terms of low-dose risk estimation, all three models have limitations. The Leung et al. (1988) model fails to reproduce the tissue concentration data from Kociba et al. (1976) and Tritscher et al. (1992). This is probably due to the high concentration of liver-binding protein (CYP1A2) predicted by this model.

The Andersen et al. (1993b) and Kohn et al. (1993) models use Hill kinetics to describe at least some of the binding reactions. Hill's equations are based on a molecular scheme of interaction in which it is assumed that there are n binding sites for the ligand and that the reaction is stable in only two states: either completely unoccupied or fully occupied. This implies that the association process is a (n+1)-molecular reaction. In the application of this model to experimental concentration curves, very little molecular meaning can be attributed to the resulting model due to the flexibility of this function with regard to dose-response shape. In addition, there is no unequivocal relationship between an estimated value of n and the existence of molecular interactions between binding sites. For example, two binding sites may exist, but binding to one site produces a small effect, while binding to both sites produces a much greater effect. This would lead to a noninteger value for n when curve fitting. Considering the importance of the Hill coefficient in terms of low-dose extrapolation (Portier et al., 1993) and considering its limitations in terms of biological understanding of the sequence of molecular events involved in induction (Andersen et al., 1993b), caution must be used when extrapolating to tissue dose regions outside of those examined directly in the experiment.

Some of the mechanistic assumptions in these models are speculative. Many of the binding and induction equations related to the Ah receptor/TCDD complex are encoded in equations, but their exact nature and level of control at the molecular level are unknown. This is true of CYP1A1, CYP1A2, the Ah-receptor, the estrogen receptor, and EGF-like peptides. Also, the reduction in EGF receptor by internalization described in the model by

Kohn et al. (1993) represents just one mechanism for its depletion. It is also possible that the synthesis or degradation of this protein may be under direct control of the Ah receptor, although TCDD does not alter mRNA levels for the EGF receptor in either human keratinocytes (Osborne et al., 1988) or mouse liver (Lin et al., 1991), and EGF receptor does seem to move from the plasma membrane to the cell interior following TCDD exposure in female rats (Sewall et al., 1993).

It should be noted that the mechanistic models have the advantage of suggesting experimental strategies for pursuing the hypothesis of action of TCDD. These models propose specific mechanisms, which can be tested in the laboratory as a means of validation of this model. For the purposes of risk estimation, one must be careful to recognize that these models do not necessarily impart added confidence in low-dose risk estimates because the mechanistic links between TCDD-mediated changes in gene expression and toxic responses are not known.

8.2. TOXIC EFFECTS

8.2.1. Modeling Liver Tumor Response for TCDD

In this section, we have decided to model liver tumor response for TCDD because of the availability of quantitative data on this end point. It should be remembered that dioxin induces increased tumor yield at several sites other than liver (see Chapter 6, Carcinogenicity of TCDD in Animals), and in humans dioxin exposure is associated with increased mortality due to lymphomas, soft tissue sarcomas, and lung and stomach cancer (see Chapter 7, Epidemiology/Human Data). We do not imply therefore by our selection of liver tumors that this is the exclusive site of dioxin carcinogenicity. It may be that similar events occur in other sites or that the events discussed below--such as estrogen interaction--are unique to rat liver.

Long-term carcinogenicity studies in rodents have shown that TCDD is a potent, complete carcinogen (Huff et al., 1991). The highest increase in yield of tumors in TCDD-treated animals as compared to controls was in female rodents. As discussed above, there is no evidence for conventional mutagenicity or DNA binding by TCDD. While TCDD clearly alters gene expression, it appears to act through a hormone-like receptor that functions as a

transcription regulator of specific genes. Moreover, TCDD clearly interacts with several endogenous hormones at the molecular and organ level. In liver, there is clearly an interaction between TCDD and estrogen. As noted above, the most sensitive animal for cancer response is the female rodent. Ovariectomy also reduced the ability of TCDD to "promote" or produce tumors in female rats pretreated with the mutagen DEN (Lucier et al., 1991). These results are complex and, as noted above, may be relevant to liver only.

Our overall approach models the tumor response observed in rodents and compares that modeled response to some of the biomarkers of exposure discussed in the section on the biochemistry of TCDD. This approach will deviate from the pure mechanistic modeling outline discussed in the introduction. Due to limitations in the data available for characterizing the models we will employ, some of the parameters used in this modeling exercise had to be obtained directly from the tumor incidence data. These parameters are then compared to the relative changes we would have expected using the biomarkers of effect and exposure that seem reasonable for this end point. Thus, this exercise falls in between curve fitting and pure mechanistic modeling. Two basic types of data will be used to find parameter estimates for the two-stage model of carcinogenesis: (1) data on the number and size of focal lesions in the liver and (2) data on the incidence of liver tumors in a 2-year feeding study.

The carcinogenicity data we will use are from a 2-year feeding study in male and female Sprague-Dawley rats (Kociba et al., 1978). For female rats, the study used 86 animals in the control group and 50 animals per group in the three treated groups given doses of 1, 10, and 100 ng/kg/day. The original pathology of the study recorded significant, dose-related increases in tumor incidence in the lung, nasal turbinates, hard palate, and liver. The original liver pathology has been reviewed several times, most recently by a group convened by Sauer (1990). The data we will concentrate on in this analysis is the incidence of liver adenomas and carcinomas (combined) based on the most recent pathology review. A summary of these data is presented in Table 8-3.

There was a substantial reduction in survival in all experimental groups (including controls) during the course of the study. Other studies have shown that correcting for this drop can result in as much as a twofold change in the low-dose risk estimates (Portier et al.,

Table 8-3. Administered Dose, Tumor Response, and Number at Risk of Hepatocellular Neoplasms in Male Sprague-Dawley Rats From Carcinogenicity Experiments of Kociba et al. (1978) Using the Pathology Review of Sauer (1990)

Administered dose (ng/kg/day)	0.0	1	10	100
Number with neoplasm	2	1	9	18[a]
Number on study	86	50	50	50
Survival-adjusted number at risk	57	34	27	31
Lifetime tumor risk	0.035	0.029	0.333	0.581

[a]All neoplasms in all groups were hepatocellular adenoma with the exception of four carcinoma in this high-dose group.

[b]Using the "poly-3" survival adjustment suggested by Portier and Bailer (1989).

1984). A simple correction for survival differences (Portier and Bailer, 1989) was applied to these data to present the risk summaries given in Table 8-3. In the analysis that follows, a more rigorous statistical approach was employed.

The data on the number and size of premalignant lesions are from an initiation-promotion study in female Sprague-Dawley rats (Tristcher et al., 1992). This study utilized 91 animals distributed into 10 groups, 5 of which received initiation doses of 175 mg DEN per kg body weight and 5 of which received 1 μL saline per gram body weight as a control. One saline group and one DEN group received no TCDD as a control for the remaining groups. Animals were exposed to TCDD by gavage once every 2 weeks in doses that averaged out to 3.5, 10.7, 35.7, and 125 ng/kg/day, respectively. Exposure continued for 30 weeks, after which the animals were sacrificed. Serial sections of the liver were stained for placental glutathione-S-transferase (PGST). PGST-positive foci were counted and their areas recorded. Table C-1 in Appendix C summarizes these data. More detailed discussions of the experimental design and focal lesion data are given in Tristcher et al. (1992) and Maronpot et al. (1993).

8.2.2. Tumor Incidence

In recent years, there has been a resurgence in interest in refining the mechanistic representation of mathematical models of carcinogenesis. With few exceptions, the mathematic modeling of carcinogenesis at the cellular level has on the use of the multistage model. Theoretical discussions on these models began in the mid-20th century (Arley and Iverson, 1952; Fisher and Holloman, 1951; Nordling, 1953). The first practical application of models from this class was done by Armitage and Doll (1954). One major failure of the Armitage-Doll model is a lack of growth kinetics of the cell populations (Armitage and Doll, 1957; Neyman and Scott, 1967; Moolgavkar and Venzon, 1979). Several researchers proposed a second model, the two-stage model, which is illustrated in Figure 8-6.

The two-stage model assumes that carcinogenesis is the result of two separate mutations, the first resulting in an intermediate cell population and the second resulting in malignancy. Cells in the normal and intermediate populations are allowed to expand in number via replication or reduce in number due to death or differentiation. There are

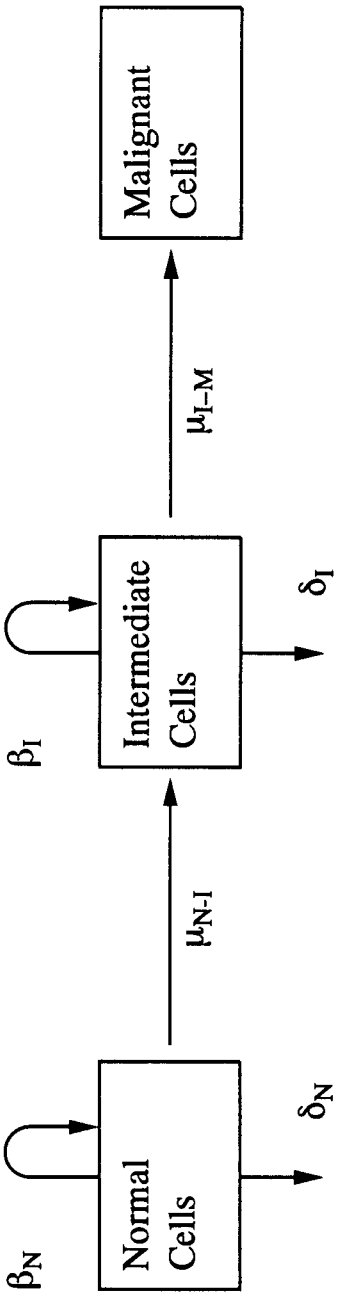


Figure 8-6. A two-stage model of carcinogenesis

different mathematical approaches to this model since several groups have proposed the same model but used different mathematical developments to predict tumor incidence from this model (Armitage and Doll, 1957; Neyman and Scott, 1967; Moolgavkar and Venzon, 1979; Greenfield et al., 1984). In the application of the two-stage model that follows, the mathematical development of this model by Moolgavkar and Venzon (1979) and subsequent development of this model will be used.

The two-stage model (Figure 8-6) has six basic rates that must be estimated. These are:

1. β_N = birth rate for cells in the normal state.
2. δ_N = death/differentiation rate for cells in the normal state.
3. $\mu_{N\text{-}I}$ = rate at which mutations occur adding cells to the intermediate state.
4. β_I = birth rate for cells in the intermediate state.
5. δ_I = death rate for cells in the intermediate state.
6. $\mu_{I\text{-}M}$ = rate at which mutations occur adding cells to the malignant state.

To apply this model to dioxin, or any other chemical carcinogen, requires estimates of these rates as they change over dose and time. A mechanistic approach to this would be to incorporate some of the relative changes in proteins seen in the NIEHS model directly into the two-stage model as rate changes in these parameters. Considering the complexity and novelty of this approach, it is left as a research topic. Instead, we will apply this model directly to tumor incidence data, focal lesion data, and cell-labeling data comparing the resulting parameter estimates to predicted dose-surrogates from the models of Leung et al. (1988), Andersen et al. (1993b), and Kohn et al. (1993).

This is not the first application of TCDD data to the two-stage model. An application of this model to TCDD was presented by Thorslund (1987). Thorslund treated the effects of TCDD as a direct promoter having an effect only on the birth rate of intermediate cells (β_I)

in the two-stage model. The number of normal cells was assumed constant (this is equivalent to setting $\beta_N = \delta_N = 0$ in the model in Figure 8-6). Two parametric models of the change in β_I as a function of dose were used, one model having a single parameter (a first-order kinetic or exponential model) and the second based upon two parameters (a log-logistic model). The parameters in the exponential two-stage model were estimated from the tumor incidence data of Kociba et al. (1978) and validated by goodness-of-fit, cell-labeling data, and species/sex/strain extrapolations. The slope parameter in the log-logistic two-stage model was chosen to be 1, 2, or 3 based on slopes observed in other biological systems. The remaining parameters in this model were estimated from the Kociba et al. (1978) data.

The liver tumor responses from the Kociba et al. (1978) study are given in Table 8-3 using the most recent pathology review of the liver sections (Sauer, 1990). Shown are the number of animals with tumor (row 2), number of animals placed on study (row 3), a survival-adjusted number of animals at risk (row 4), and the survival-adjusted lifetime tumor probability (row 5 which equals the entry in row 2 divided by the entry in row 4).

There are a variety of mathematical formulations that could be used to derive a tumor incidence rate under the two-stage model. As has been assumed by other authors (Portier and Kopp-Schneider, 1991; Moolgavkar and Luebeck, 1992), we assume:

1. All cells act independently of all other cells;

2. The rates in the two-stage model (Figure 8-6) are constant over the lifespan of the animal;

3. The tumor incidence rate corresponds to the rate of appearance of the first malignant cells; and

4. $\beta_N = \delta_N = 0$ (no change in the number of normal cells).

All four of these assumptions are likely to be violated for most chemicals. In most tissues, there is a homeostatic feedback system to control the number of cells in the tissue. No such system can be assumed here since it results in a mathematic formulation that is either intractable or has yet to be developed. For the large pool of normal cells in the liver,

this is unlikely to have an effect, but for the small number of intermediate cells (at least for early times), this could have a small effect on tumor incidence. This issue cannot be resolved without further research. Assumption (2) is clearly violated based on the behavior of the PB-PK models presented earlier. Time-dependent changes in tumor incidence could be incorporated into the modeling and will be at a later time. Finally, the kinetics of cell growth for malignant clones have been studied (Moolgavkar and Luebeck, 1992), and assumption (3) was found to have a moderate impact on the tumor incidence rates. This will be investigated further at a later time.

The exact tumor incidence rate was used to avoid potential bias from the routinely used approximation (Kopp and Portier, 1989). Thorslund (1987) used this approximation in his analysis. The methods outlined by Portier and Kopp-Schneider (1991) employing the Kolmogorov backwards equations were used to derive the exact tumor incidence rate.

It has been suggested that the cells in PGST-positive lesions correspond to the initiated mutated cells in the two-stage model of carcinogenesis. In the following analyses, we have assumed this to be the case, but it is important to keep in mind that the number of enzyme-altered cells may not correspond to mutation rate. If this is true, it is possible to apply the methods of DeWanji et al. (1989) to data from Lucier and colleagues (Tristcher et al., 1992) to analyze the growth characteristics of these cells (Moolgavkar et al., 1990). This can be combined with the tumor incidence data to produce a combined model that explains both sets of data. This is done below. Analyses of the tumor incidence data alone and the PGST-positive foci data alone are given in Appendix C.

The most parsimonious two-stage model that agrees with the tumor incidence data and the focal lesion data (this is based on a combination of likelihood ratio testing and plots of the model versus the data) is a hyperbolic function in dose for the effect of TCDD on the rate of transformation from the normal state to the intermediate state, is hyperbolic in dose for the birth rate of intermediate cells, is constant in the ratio between the death rate and the birth rate for intermediate cells, and uses a power function in dose on the rate of mutation from the intermediate state to the malignant state. The resulting model is given as:

$$\mu_{N\text{-}I}(\text{dose}) = \mu_0 + \frac{\mu_V \text{dose}}{\mu_k + \text{dose}} \quad [4]$$

$$\beta_I(\text{dose}) = \beta_0 + \frac{\beta_V \text{dose}}{\beta_k + \text{dose}} \quad [5]$$

$$\delta_I(\text{dose}) = \rho\beta(\text{dose}) \quad [6]$$

$$\mu_{I\text{-}M}(\text{dose}) = \mu_2 + \mu_3\ \text{dose}^{\mu_4} \quad [7]$$

where $x_0\ \mu_0 = 3.216 \times 10^{-4}$, $x_0\ \mu_v = 6.856 \times 10^{-4}$, $\mu_k = 19.75$, $ß_0 = 3.029 \times 10^{-2}$, $ß_V = 4.296 \times 10^{-3}$, $ß_k = 2.75$, $\rho = 0.00302$, $\mu_2 = 1.496 \times 10^{-7}$, $\mu_3 = 3.082 \times 10^{-9}$, $\mu_4 = 2.56$, and dose is expressed in ng/kg/day. This analysis also assumes that the average rodent liver has 6×10^8 normal hepatocytes. The fit of this model to the number and size of focal lesions from the studies of Lucier et al. is illustrated in Figures 8-7 and 8-8. It is clear from these plots that this fit is adequate; this is supported by analyses based on the magnitude of the likelihood (see Appendix C). The fit of this model to the tumor incidence data of Kociba et al. (1976) (PWG, 1990) is shown in Figure 8-9.

It is possible to compare the parameters estimated for the two-stage model to predictions from the PB-PK models to try to locate a reasonable mechanistic link between the two classes of models, to aid in species extrapolation, and to help guide us in choosing the most appropriate curvature for low-dose extrapolation. An overall comparison of the dose surrogates is given in Appendix D. What is illustrated in Appendix D is that most reasonable dose surrogates correlate well with the dose-response relationships given above and in Appendix C. For the purposes of finding a most reasonable model for low-dose risk assessment, we will use two specific dose surrogates from Appendix C.

In the NIEHS PB-PK model (Appendix A), it is hypothesized that induction of CYP1A2 could lead to an increase in the metabolism of estrogens to catechol estrogens

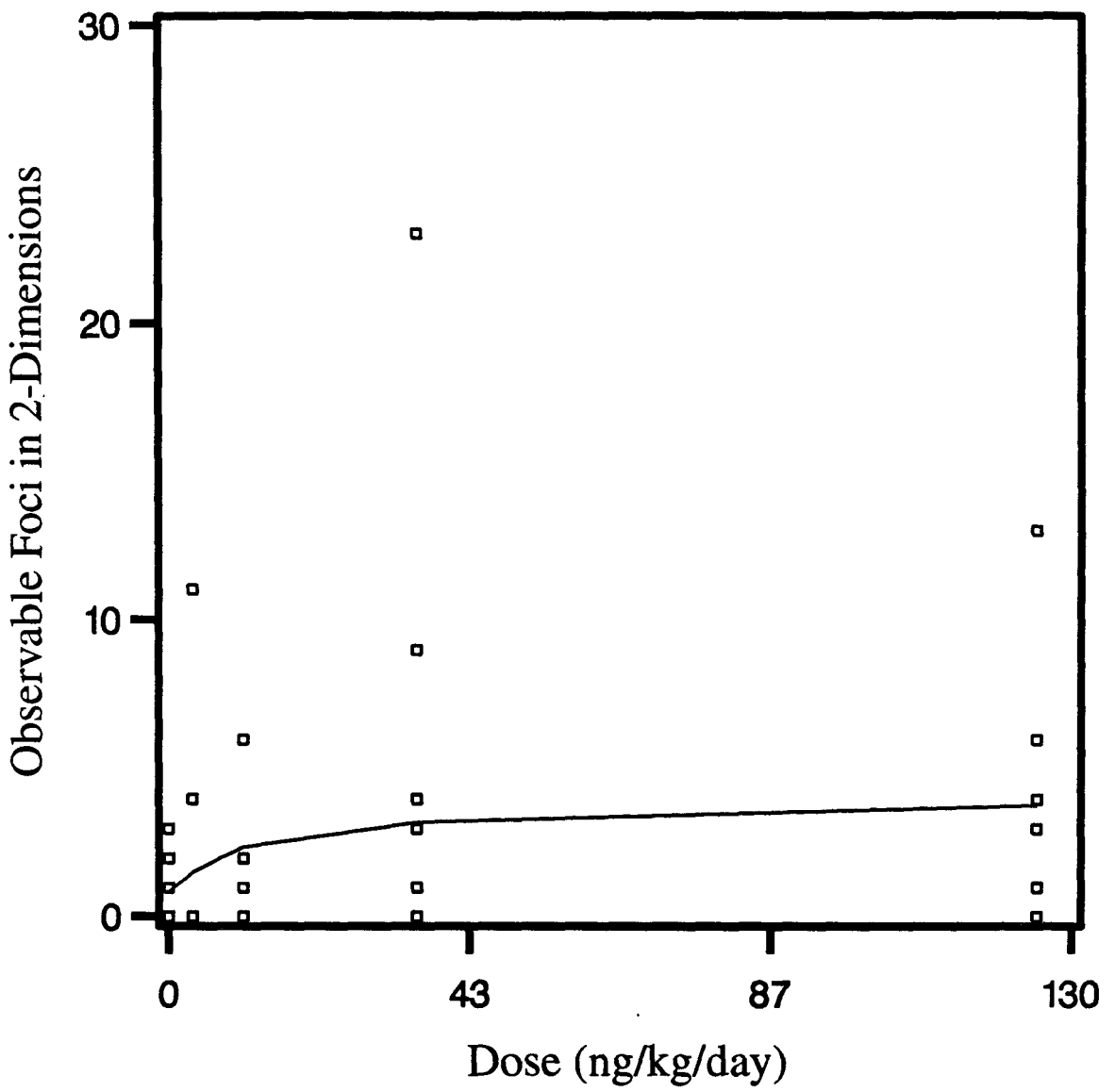


Figure 8-7. Fit of the two-stage model to the number of focal lesions from the data of Maronpot et al. (1993).

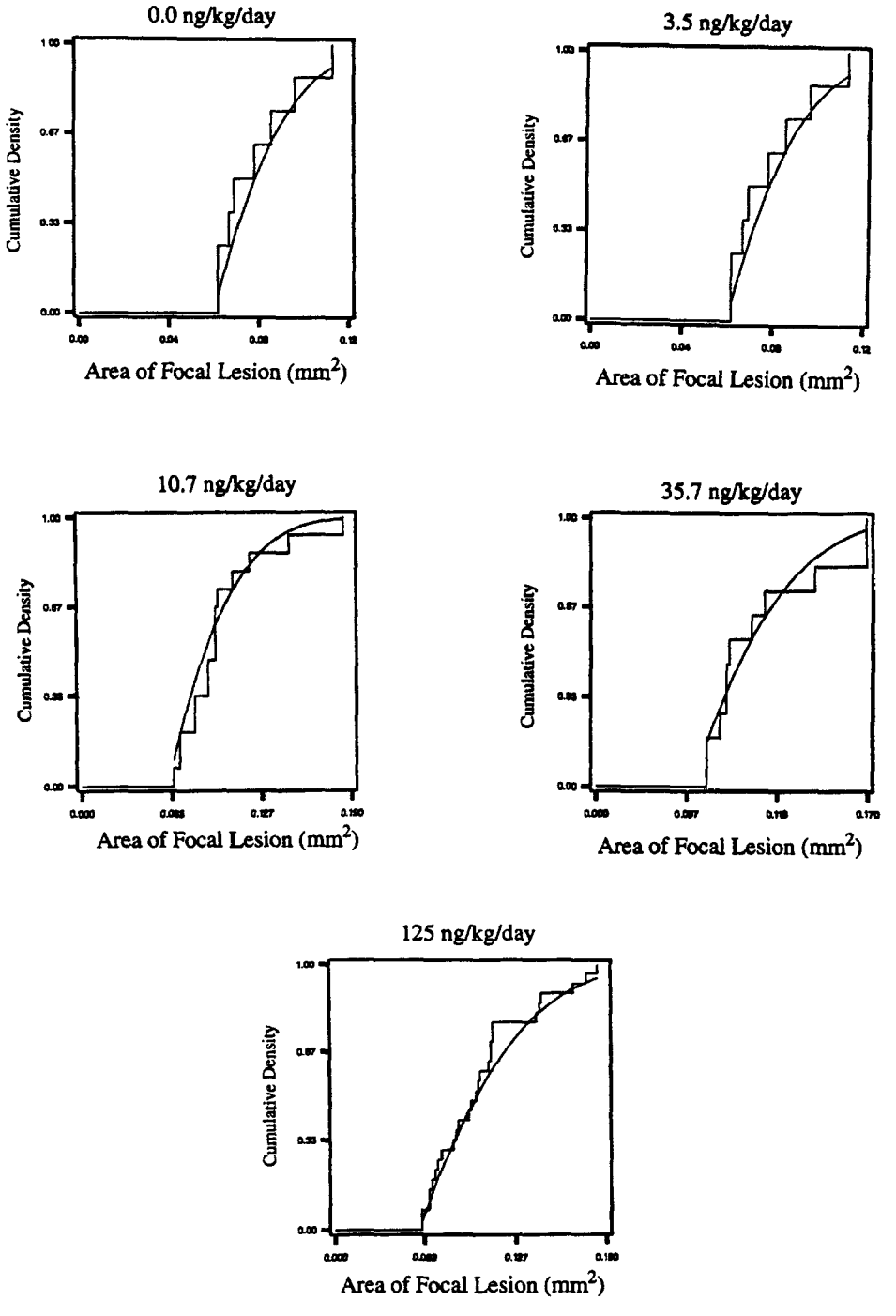


Figure 8-8. Fit of the two-stage model to the size distribution for focal lesions from the data of Maronpot et al. (1993) where the smooth line is derived from the model and the step function results from the data.

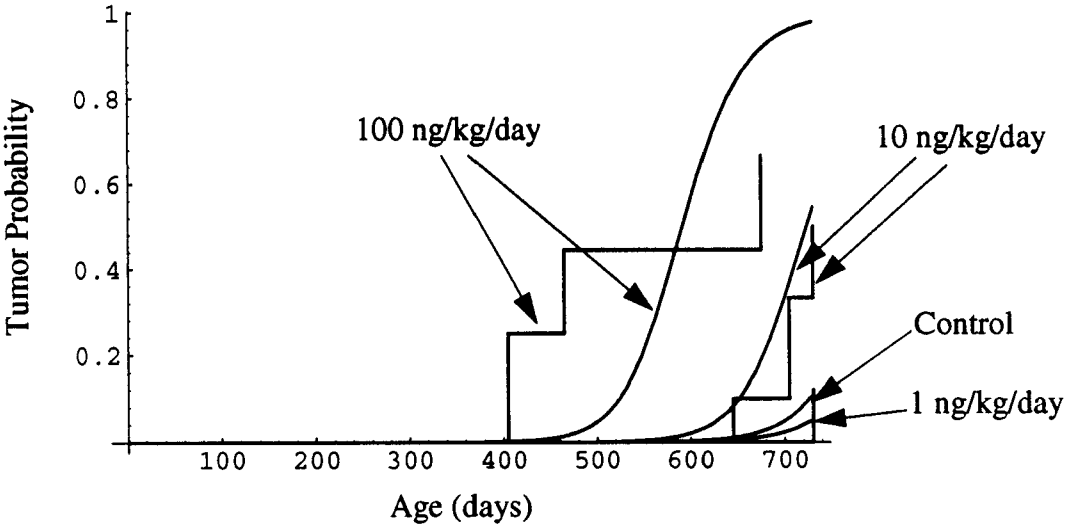


Figure 8-9. Fit of the TS model to the data of Kociba et al. (1978) using parameters for $\mu_{N\text{-}I}(d)$, $\beta_I(d)$, and $\delta_I(d)$ estimated from the focal lesion data.

(Graham et al., 1988) and that further activation of these catechol estrogens can lead to cell damage (for example, via free oxygen radicals) and eventually to mutations. Thus, the area under the time-concentration curve for induction of CYP1A2 could serve as a useful dose surrogate for the mutational effects of TCDD expressed as equation (4). Note that this relationship is speculative and that the application of dose surrogates to low-dose risk estimation is subject to a number of untestable assumptions. This is discussed in detail in Appendix D.

In this case, the shape of the response for the area under the time-concentration curve for the induction of CYP1A2 parallels the hyperbolic effect given above. A better approach to this analysis would be to use the instantaneous concentration of CYP1A2 as it changes over time to directly modify the mutation rate. However, at this time, this approach is impractical due to inadequate mathematical development and a lack of computer algorithms and programs for addressing the problem. However, the parallel curves for CYP1A2 and the mutation rate indicate that dramatic changes in low-dose response by going to the time-varying dose surrogate is unlikely.

Kohn et al. (1993) also provided a potential mechanism for the proliferative effects of TCDD on the cells in the intermediate state. For this process, they propose a mechanism based on the incorporation of the EGF receptor in an activated state in the cell interior rather than on the cellular membrane. However, the relationship between the area under the time-concentration curve for the internalized EGF receptor does not correlate well with the hyperbolic dose response seen in the growth characteristics of the intermediate cells in the two-stage model given by equation (5) above. This could be due to a number of factors: incorrect two-stage model specification, incorrect specification in the NIEHS model, or a complex relationship between internalized EGF receptor and mitosis of the intermediate cells. Either way, the simple dose surrogates expressed in Appendix D are inappropriate for the parameterization of the two-stage model given above. Note that for both the mutation rate and the mitotic rate, dose-response behavior is adequately described by a linear function in the low-dose region.

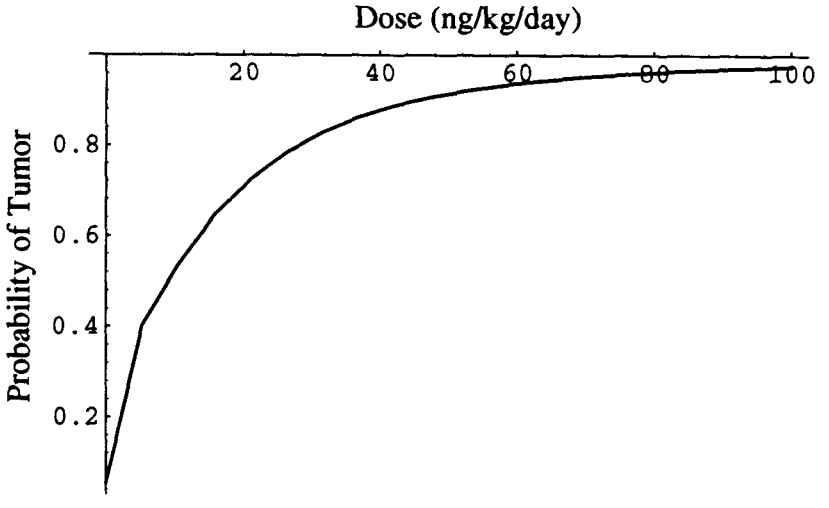
The details of a complete analysis of the two-stage model and the Kociba et al. (1978) data are given in Appendix C. The fit of the model given by (7) above to the data by Kociba

et al. (1978) is shown in Figure 8-9. The fit is adequate and is not statistically significantly different from the best-fitting two-stage model, which is nonparametric for $\mu_{I\text{-}M}(d)$ and uses the parameters derived from the focal lesion analysis. It is significantly different from the best-fitting two-stage model in which all parameters are derived from the tumor incidence data alone (see Appendix C).

Because $\mu_4 > 1$, the relationship between dose and the mutation rate between the intermediate state and the malignant state is sublinear (similar to threshold behavior). As discussed in Appendix D, none of the dose surrogates for the NIEHS model exhibit this type of behavior. In Andersen et al. (1993b), induced CYP1A1 has this type of behavior (this is also seen in Portier et al., 1993). The difference between the Andersen et al. findings and those of Kohn et al. (1993) are discussed in Appendix D and will not be repeated here. Also, as explained in Appendix D, these correlations provide very little verification of an actual mechanistic link between the dose surrogate and the tumor incidence rate.

Even with this nonlinearity in $\mu_{I\text{-}M}(d)$, the dose response for lifetime tumor incidence is linear in the low-dose region (Figure 8-10). This is due to the linear effect of dose on both $\mu_{N\text{-}I}(d)$ and $ß_I(d)$ in the low-dose region. In general, with these two-stage models, if any rate in the process is linear and all other rates are nondecreasing, the low-dose behavior of the model will be linear. Extrapolation to low doses can be accomplished directly by using the parameters described above or through the PB-PK models described in the previous section. In order to utilize the PB-PK models, a dose surrogate must be chosen for a particular rate (parameter) in the two-stage model. To do this, one would proceed as follows. Let's denote the level for a dose surrogate and define f(d) to be the functional relationship between dose (d) and the dose surrogate (s). If f is 1-1 and onto, then the inverse equation, f^{-1}, exists and $d = f^{-1}(s)$ can be used in the two-stage model above. For example, suppose a reasonable dose surrogate for the effect of dose on $\mu_{N\text{-}I}$ is the induction of CYP1A2. Let $c_A(d)$ denote the relationship between dose and induction of CYP1A2 in the test animal. Instead of using $\mu_{N\text{-}I}(d)$ for extrapolation and modeling, one could use $\mu_{N\text{-}I}(s)$, having first converted all administered doses into the dose surrogate, s, using $C_A(d)$. Extrapolation would then be done in units of CYP1A2 induction, and extrapolation would result in a predicted induction of CYP1A2, say ϵ_A. To estimate exposure doses in humans

Part A: Observable response region



Part B: Low-dose region

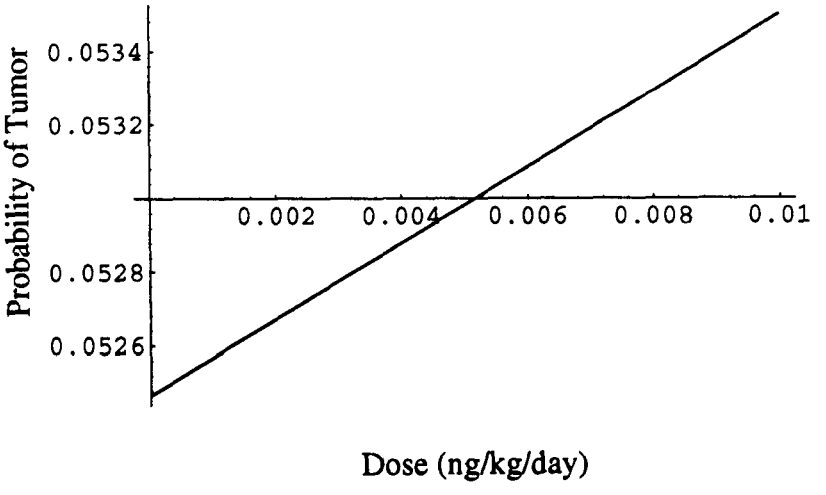


Figure 8-10. Dose-response for tumor incidence using a two-stage model of carcinogenesis.

would then require knowledge of the induction of CYP1A2 by TCDD in humans, say $s_H=C_H(d)$. The predicted human exposure dose (presence of induction) would be $d_H=C_H^{-1}(\epsilon_A)$. It is important to reemphasize here that enzyme induction is not equated to toxic response.

When multiple dose surrogates are used, the equations become more difficult due to the multivariate nature of the relationship. For example, if we were using CYP1A2 induction as a dose surrogate for $\mu_{N\text{-}I}(d)$ and removal of EGF receptor from the plasma membrane as a dose surrogate for $ß_I(d)$, our functional forms would have to be concerned with the changes in both of these proteins as a function of dose. However, the basic idea remains the same, and inversion of the formulae is used to estimate safe exposure levels. Since there are, as yet, no comprehensive PB-PK models for humans, this approach could not be used here. Instead, the results of the PB-PK model are used to support dose-response relationships seen for the tumor incidence data and the focal lesion data.

The final model resulting from the combined analysis of the tumor incidence data and the focal lesion data has several shortcomings. One serious problem concerns the size of the intermediate cell population after 2 years of exposure to TCDD. The model does an excellent job of describing the size and number of focal lesions in the liver at 31 weeks; but, by 2 years, since the same rates seen in the first 31 weeks are assumed constant for 2 years, the model predicts impossibly large foci in the liver (the model predicts that the mean number of intermediate cells is two times larger than a normal rodent liver in the high-dose group). The data available for this analysis consist of single time point data so there is no data-driven way in which to estimate changes in the model parameters (mutation, birth and death rates) over time. Arbitrary changes could be used; however, these are unlikely to change the shape of the dose-response curve for $\mu_{I\text{-}M}(d)$ as long as the changes are applied proportionately across all treatment groups.

Additional data will be required to support any improvements in this model. Additional focal lesion data (size and number) at a time point other than 31 weeks would be very useful in terms of looking at time-dependent changes in the rates in this model. Considering the size and number of saline-treated animals with foci at 31 weeks, it would probably be best to have information on focal lesions at a later time. Because the

proliferation rates from the focal lesion data match the observed labeling index data from Maronpot et al. (1993), the simplest change that will lead to reasonable numbers of initiated cells is a change in the ratio of the death rate to the birth rate for initiated cells. As the animal ages, this rate will have to converge to 1 or less for the expected number of initiated cells to have a reasonable size at 2 years. If the change over time is proportionately the same over time in all dose groups, it should have little or no effect on the shape of $\mu_{I\text{-}M}(d)$; the only effect should be on the magnitude of this mutation rate (increasing it). Any dose-dependent changes in this ratio may alter the dose-response shape of $\mu_{I\text{-}M}(d)$ and hence also alter the low-dose estimates of risk.

One final note on the final version of the two-stage model fit to the tumor incidence data and the focal lesion data: In the low-dose region, the dose-response curve for added risk from exposure to TCDD can be approximated by the formula risk=0.024 dose where dose is in units of ng/kg x bw/day. The formula can be inverted to estimate a safe exposure dose from these data. For an added risk of 1 in 1 million in the animals, the calculated exposure dose is 41.7 fg/kg x bw/day. This is a point estimate of exposure dose; confidence intervals would reduce this value.

We have described modeling as a continuing process. One must remember that, as a continuing process, modeling allows us to test certain hypotheses developed from a particular model in hopes of designing experiments that can either support or refute the model. Modeling also allows us to determine how well a particular hypothesis, derived from biological evidence, can explain the observed phenomena. One of the models presented here has analyzed two data sets and has presented a hypothesis that dioxin increases, in a dose-dependent manner, the rate at which normal cells become initiated cells, thereby increasing the number of initiated cells. If the model is correct, then dioxins are acting to increase the mutation rate in hepatocytes, thus increasing the number of initiated cells. In essence, dioxins are acting as indirect mutagens.

The impetus in developing a biologically based dose-response model for dioxin was that there is no evidence that dioxins are direct mutagens and that the carcinogenic actions of dioxins were due to their actions as receptor-mediated tumor promoters. In theory, tumor promoters do not alter the mutation rate and act only on previously initiated cells. In

addition, the actions of a tumor promoter are reversible. If we compare this theory to that of the model's prediction of dioxin's actions, we are left with several contradictions. Either the structure of the model has led us astray or the actions of dioxins increase the mutation rate through indirect mechanisms and that these effects are not detectable in short-term assays.

There is consensus that dioxins are not directly mutagenic, and there are very few data that demonstrate that dioxins damage DNA directly and/or are mutagenic in short-term assays. (See Chapter 6 for detailed discussion.) However, a recent study suggests that the short-term assays may not be sensitive to the potential indirect mutagenic effects of dioxins. Yang et al. (1992) have reported that exposing nontumorigenic immortalized human keratinocytes to TCDD for 2 weeks transforms these cells into tumorigenic cells. Exposures for less than 2 weeks do not result in transformation of this cell line. These experiments indicate that exposure to dioxins can result in permanent changes in the phenotype of human cells. While the mechanism by which this occurs is undetermined, it is clear that these permanent alterations require prolonged exposures in order to be detected. These permanent changes are contradictory to the hypothesis that dioxins act solely on the promoter stage in the process of carcinogenesis.

If dioxins were to increase mutation rates through indirect mechanisms, what actions of dioxins would increase the likelihood of a mutational event? One possibility is that by increasing CYP1A1 and CYP1A2 activity, dioxins increase the production of reactive intermediates. Evidence to support this hypothesis is that CYP1A2 metabolizes estradiol to the catechol estrogen 2-hydroxyestradiol. Catechol estrogen formation may lead to DNA damage and may be responsible, in part, for the carcinogenic effects of estrogens.

Dioxins may also have indirect mutagenic actions through increased hepatocyte proliferation. Cells are constantly exposed to reactive intermediates that may cause DNA damage. Inappropriate stimulation of cell proliferation, by dioxins, may produce an apparent increase in the number of initiated cells in a tumor promotion study by forcing cells with nonlethal DNA damage to replicate prior to the repair of the damage. This could lead to fixed mutations in the genome and clonal expansion of the transformed cells, resulting in an increase in preneoplastic foci.

Increased transcription of a gene can also lead to potential mutagenic events. Glucocorticoids can produce a variety of genetic rearrangements through epigenetic mechanisms. If cells transfected with a plasmid containing a glucocorticoid response element and a recorder gene are exposed to dexamethasone, a variety of mutagenic events such as inversions, deletions, and insertions in the plasmid occur. In addition, antisense mRNA can also be detected following exposure to dexamethasone. This study demonstrates that increased transcription of the recorder gene by dexamethasone increased the likelihood of a mutational event in the recorder gene through epigenetic mechanisms. Dioxins act in a similar manner to dexamethasone and other steroid hormones. Both classes of chemicals bind to intracellular receptors, and the activated receptor binds to response elements on DNA, resulting in an increase in gene transcription. It is possible that continued exposure to dioxins will increase the likelihood of a mutagenic event at a gene that is responsive to dioxins due to the increased rate of transcription. Since dioxins alter growth properties of a variety of different cell types such as hepatocytes (Lucier et al., 1991), skin (Stohs et al., 1990), and embryonic palate (Abbott and Birnbaum, 1991), it is possible that some of the genes that can be affected by this mechanism would be involved in the regulation of cellular proliferation.

Chemical carcinogenesis is often divided into stages of initiation, promotion, and more recently progression (reviewed in Pitot and Dragan, 1991). As the process of carcinogenesis develops, the progression stage is an irreversible stage that is associated with increase in karyotypic instability, which is associated with the development of aneuploid malignant neoplasms (Pitot and Dragan, 1991). Thus, during the carcinogenic processes there are steps postinitiation that result in karyotypic instability. It is possible that dioxins are acting not only as promoters but also as progressors, and the mechanisms described above may provide some insight into how dioxins act.

8.2.2.1. *A Potential Alternative Model for Promotion of Carcinogenesis by Dioxin*

PB-PK models permit estimation of tissue concentrations of dioxin and of various protein products regulated either directly or indirectly by dioxin and the Ah receptor. In developing a quantitative mechanistic model of the tumor responses to dioxin, it is necessary

to propose some relationship between the concentration of these protein factors and the critical cellular responses—mutation rates and cell proliferation rates—leading to tumor induction. One hypothesis, consistent with dioxin's primary mechanism as a promoter, is that dioxin, directly through effects on mitostimulatory hepatic growth factors, such as transforming growth factor-α, causes enhanced cell replication in normal and/or initiated cells. In some of the cancer modeling in this chapter, this approach has been followed at least indirectly by assuming that the reductions in cell surface EGFR concentrations are an indication of enhanced levels of an activating ligand for this receptor and that the reduction in EGFR is a surrogate for increased cell proliferation rates.

Pitot et al. (1987) examined promotion of altered hepatic foci by dioxin in rats that were initiated with a DEN dose of 10 mg/kg following 70% partial hepatectomy. These altered hepatic foci are believed to be precursor lesions for overt hepatic malignancies. In this study, doses used were equal to the bioassay doses used by Kociba et al. (1978) with an additional low dose, 0.0001 μg/kg/day. One important aspect of this study and the original dioxin bioassay is the dose range covered, 100-fold in the cancer bioassay and 1,000-fold in the promotion study. Pitot et al. (1987) found that the promotion dose-response behavior was U-shaped; the two lower doses gave a lower response for volume of liver as foci or total number of observed foci than did either the control or high-dose groups. This U-shaped dose-response behavior was also observed in the liver tumor incidence in the bioassay study, although the differences between control incidence and incidence at the lower doses were not statistically significant. These observations indicate a more complex relationship between dioxin exposure and response than expected for dose surrogates, whether dioxin concentrations, cellular EGFR concentrations, or protein product concentrations, that increase (or decrease) monotonically with dioxin concentration.

Although less marked than with dioxin, phenobarbital also had a U-shaped dose-response behavior for hepatic promotion when examined with the same experimental protocol over a dose range of 0.001 to 0.05% in the diet (Pitot et al., 1987). Jirtle and coworkers (1991) have examined the biology of phenobarbital promotion in detail. They propose that phenobarbital initially causes a mitogenic stimulus with proliferation of pericentral hepatocytes. The liver responds to the persistent proliferative signal by increasing levels of

mitoinhibitory growth factor, TGF-β1, and periportal hepatocytes stain intensely for TGF-β1. Down-regulation, presumably without activation, of EGFR also occurs in phenobarbital-treated livers. The hallmark of promotion in this hypothesis is the selection provided by the persistent increases in TGF-β1 for initiated cells with particular alterations that decrease their responsiveness to these inhibitory growth factors. Consistent with this mechanism, the putative preneoplastic hepatocytes express significantly less TGF-β1 than do the periportal hepatocytes (Jirtle and Meyer, 1992). The suppression in growth of the normal cells in the tissue then is the critical alteration leading to promotion and really is more of a homeostatic response than a direct response of the cell to dioxin. In this model of hepatic promotion, the increases in mitoinhibitory factors serve to maintain the liver at constant size even in the continued presence of proliferative stimuli provided by dioxin, phenobarbital, or other xenobiotics that might share similar mechanisms of action. The clonal expansion of initiated cells results from a growth advantage because of their relative insensitivity to the antiproliferative environment produced by the promoter (Jirtle et al., 1991).

This general mechanism of promotion may also occur with dioxin (Mills and Andersen, 1993) and could give rise to the U-shaped dose-response curve seen by Pitot et al. (1987). Initiated cells might have differential sensitivity to apoptosis mediated by TGF-β1 (Oberhammer et al., 1992) and to proliferation induced by other factors regulated by dioxin. If the apoptotic rates were increased at lower dose than doses that enhance proliferation, a U-shaped curve would be expected. Another possibility is that clones observed in control (PH/DEN; 0 μg dioxin/kg/day) rats are derived from different precursor cells than are those clones arising in livers of rats treated with higher daily doses of dioxin (>0.01 μg/kg/day). In the control rats no net increase in TGF-β1 is expected. The initiated cells that become foci in these rats are potentially responsive to the antiproliferative activity of TGF-β1 and, as the dose of dioxin is increased, the death rates of these cells increase; fewer clones grow out to become observable foci, and a lesser volume of the liver is occupied by these clones. At higher dioxin doses, tissue TGF-β1 is increased and the selection environment favors growth of a new population of initiated cells that are TGF-β1 nonresponsive. At intermediate doses, the primary response is inhibition of the first type of initiated cells with a decrease in yield of observable clones. At these doses there is not yet any increase in the appearance of

clones arising from the second population of initiated cells. This behavior would lead to a U-shaped dose-response curve. Early efforts to parameterize these promotion models (Andersen et al., 1993a) indicate that the model assuming a different cellular origin of clones in control versus high-dose dioxin rats appears the more likely of these two hypotheses at this time.

8.2.3. Other Effects: Mammary/Uterine/Anticancer End Points

There are several lines of evidence that suggest that TCDD is anticarcinogenic in some organs of experimental animals. The most compelling data are from the Kociba bioassay (Kociba et al., 1978), which reported a dose-dependent decrease in several endocrine tumors in females. The most remarkable changes occurred in the incidence of carcinoma of the breast and uterus and all tumors of the pituitary. The decrease in these tumors coincided with the increase in hepatic tumors in the same animals. Tumor inhibition has also been reported in skin initiation-promotion experiments if TCDD administration precedes the initiator. This TCDD-induced inhibition occurs even if the complete initiator-promoter protocol is carried out following the TCDD administration (DiGiovanni et al., 1977; Marks et al., 1981).

Mechanistic explanations for these observations are incomplete. Several laboratories have attempted to elucidate the molecular events leading to the decrease in tumors particularly in the breast and uterus. Three lines of evidence are under great scrutiny at this time, including (1) alteration of the estrogen receptor, (2) alteration of estrogen metabolism, and (3) alteration of binding of estrogen and estrogen receptor at the DNA level.

Down-regulation of the estrogen receptor has been reported by laboratories of Gallo (Gallo et al., 1986; DeVito et al., 1990), Safe (Romkes et al., 1987; Astroff and Safe, 1988), and Gierthy et al. (1988). This down-regulation occurs in a rank order relationship with the affinity of TCDD structural analogs to bind to the Ah receptor (Harris et al., 1990) and parenthetically with the ability of the compounds to induce cytochrome P-450IA1. There is differential regulation of the estrogen receptor (ER) by TCDD in uterus and liver (liver being more sensitive), and the regulation of the uterine ER disappears in the adult animal (DeVito et al., 1992). Collectively, these observations suggest that there are at least two

mechanisms involved in ER regulation by TCDD. Gierthy has supported the argument that, in MCF-7 cells, the alteration of estrogen metabolism following the induction of cytochrome P-450 isozymes is the explanation for the decreased activity of the ER. Safe has reported that the ED_{50} for decreased ER in MCF-7 mice cells is several orders of magnitude below the ED_{50} for induction of 7-ethoxyresurofin-O-deethylase (EROD) in these same cells (Zacharewski et al., 1991). In vivo studies suggest that the uterine response is more responsive to changes in circulating estradiol (which may or may not hold up for breast tissue), but the hepatic ER response to TCDD is independent of circulating estradiol levels (DeVito et al., 1992). Another possibility is that TCDD induces enzymes that qualitatively change in situ estrogen metabolism and that these novel metabolites (or families of metabolites) alter estrogen action. Recent evidence suggests the presence of a 15-alpha metabolite of estradiol in liver microsomes, from TCDD-treated females, incubated with estradiol. This observation is similar to that of Gierthy et al. (1988) in MCF-7 cells.

Another possible mechanism of the decrease in breast cancer is that the binding of the activated AhR complex to a DRE on the DNA is in the upstream region of the ER gene or is in the vicinity of the binding region of the activated ER. Several lines of evidence also support this hypothesis since there is ample evidence that other steroid receptor complexes modify the DNA binding of different members of the steroid receptor superfamily (Gustafsson et al., 1987; Cuthill et al., 1987).

The last area to be discussed here is the role of the pituitary in the regulation of the endocrine system and in particular the relationships between pituitary-hypothalmus and the estrogen receptors. The ER in liver and uterus is controlled by the pituitary (Lucier et al., 1981), and growth restores the albation of hypophysectomy. Hypophysectomy has little effect on TCDD induction of P-450, but TCDD does decrease ER in growth hormone-restored animals (DeVito et al., 1991). Interestingly, Peterson et al. (See Chapter 5, Developmental and Reproductive Toxicity) have made similar observations for regulation of testosterone and testicular function by TCDD.

The importance of this subsection is at least twofold. First, the regulation of the estrogen receptor is one of the most sensitive non-P-450 markers of TCDD exposure in immature females, and this regulation coincides in rank-order fashion with decreases in

mammary, uterine, and pituitary tumors in TCDD-treated female Sprague-Dawley rats. Second, the removal of the ovaries (the primary source of estradiol) inhibits the formation of hepatic tumors in rats by TCDD (Lucier et al., 1991) without altering the ability of TCDD to induce cytochrome P-450IA1 (DeVito et al., 1992). However, ovariectomized animals developed lung tumors in a two-stage model in rats, whereas no lung tumors were detected in intact rats subjected to the same two-stage model (Clark et al., 1991). Taken together, these findings indicate that estrogens can either enhance or protect against TCDD-mediated cancer in a site-specific manner and that there is likely more than one mechanism for TCDD's carcinogenic action.

8.2.4. Noncancer End Points

Previous risk assessments have focused primarily on cancer as the most important and sensitive end point. This assumption has recently been questioned. For example, lead is carcinogenic in experimental paradigms, yet it is the neurotoxicity that drives the risk assessment. Past risk assessments of TCDD and its congeners have also focused on cancer as the primary toxic end point, although it produces adverse effects in a wide variety of tissues and cells. It is possible that the immunological, reproductive, or developmental toxicities of TCDD are just as sensitive and important in the risk assessment process. For noncancer end points, risk assessments traditionally have used the safety factor method to estimate risk. Biologically based mathematical models for noncancer end points have not been extensively utilized and are not as developed as are cancer risk models. The development of biologically based models requires that the responses are well characterized, tissue doses have been established, and sufficient data are available to propose a mechanistic model. Many of the toxic effects of TCDD are well characterized with respect to dose-response relationships, time-course relationships, species differences, and the magnitude for the effects. Qualitative and quantitative evaluation of dose-response relationships for noncancer end points is presented in Appendix E. This is a valuable exercise because it looks at consistencies and inconsistencies among species strain and exposure regimen. It helps to identify data gaps and provides a road map for future studies that will enable biologically based risk assessments for noncancer end points. The development of

biologically based mechanistic models requires more extensive data sets on the putative mechanisms through which TCDD produces its toxic effects. However, it is important to note that many of the same molecular events involved in TCDD-mediated cancer may also be involved in the production of noncancer end points such as alterations in transforming growth factor-2 (TGF-2), EGF receptor, and estrogen receptor. Therefore, as we learn more about the mechanisms of TCDD-mediated, noncancer effects, we may be able to readily apply cancer mechanistic models to other toxic effects.

8.2.5. Neurological and Behavioral Toxicity

The neurotoxic effects of the dioxins and related compounds have not received much attention, in comparison to other target organs, despite a number of clinical and semianecdotal reports of neurotoxic signs and symptoms in exposed humans (see, for instance, Ashe and Suskind's (1985) reports on the Monsanto workforce; Jirasek et al., 1974; Poland et al., 1971). There are some reports on neurochemical changes in animals associated with exposures to PCBs and phenoxyacetic acids (Tilson et al., 1979). PCB exposure also induced motor dysfunctions in some but not all mice (Tilson et al., 1979), suggestive of effects on basal ganglia (circling and spinning).

Recently, Seegal and coworkers (1990) have reported significant effects of certain PCBs on brain chemistry, specifically on aminergic pathways (norepinephrine, dopamine, and serotonin). The structure-activity relationships of these effects suggest that they are not associated with the Ah receptor, since it is the noncoplanar, low-chlorinated, nondioxin-like PCBs that are neuroactive. These results are consistent with a report by Silbergeld (1992) that the Ah receptor was not detected in neurons although it was measurable in glia.

Dioxins may be neurotoxic through indirect actions that affect nervous system function and development. Some of the most exciting information in this area has been published by Peterson and coworkers. They have reported that very low level, single-dose exposures of pregnant rats results in offspring with significant alterations in sexual behavior, characterized as a feminization of male rats (Mably et al., 1992a, b, c). It is well known that the fetal endocrine milieu is critical to the development of the mammalian brain, particularly the sexually dimorphic nuclei of the hypothalamus and cortex (Becker et al.,

1992). There are critical periods of vulnerability during which small alterations in levels of testosterone during gestation have profound and longlasting effects on brain and behaviors. Mably et al. (1992a, b, c) and Peterson et al. (1992) have reported that these prenatal exposures to dioxin result in altered patterns of sexual behaviors in male rats. It is likely that the behavioral effects in this model are consequent to endocrine effects that impair the sexual "imprinting" of the brain, rather than direct neurotoxic actions of dioxin on the developing brain. Whether females are as sensitive to these intrauterine effects is not presently known; since the male must be differentiated over embryofetal development from the primordial female phenotype, it is possible that the male is more sensitive to these endocrine-mediated effects. The consequences of these exposures for behaviors other than sexual are also not known; however, other studies indicate that alteration in intrauterine imprinting can affect motor activity and learning in males. These findings open up important new areas of toxicological research on the dioxins.

8.2.6. Teratological and Developmental

8.2.6.1. *Cleft Palate*

Dioxins produce structural malformations and developmental toxicity in several species. Considerable information is becoming available on mechanisms of cleft palate formation, and it may be possible to construct mechanistic models for this effect. In mice, increases in the incidence of cleft palate are well-characterized phenomena (Birnbaum et al., 1987a, b, 1991). The doses required to produce cleft palate in mice are well below doses that produce maternal toxicity or fetal mortality. In the normal developing palate, the peridermal medial epithelial cells cease to express EGF receptor, decrease cell proliferation, and eventually undergo programmed cell death while the basal cells differentiate into mesenchyme, allowing the left and right palate to fuse. Temporal changes in the expression of EGF receptor, EGF, TGF-alpha, TGF-beta 1, and TGF-beta 2 are critical for the fusion of the palate. Experimental evidence indicates that changes in expression of these factors, induced by TCDD, results in cleft palate formation. The medial epithelial cells of cultured mouse embryonic palates exposed to TCDD express EGF receptor, incorporate [^{3}H]-thymidine, and differentiate into a stratified squamous oral-like epithelium in a dose-

dependent manner (Abbott and Birnbaum, 1989). Changes in medial epithelial cell differentiation are associated with increased EGF receptor, TGF-beta 1, and TGF-beta 2 and decreased TGF-alpha levels (Abbott et al., 1992).

The use of cultured embryo palates (Abbott and Birnbaum, 1991, 1990a; Abbott et al., 1989) has (1) led to a greater understanding of the mechanism of TCDD-induced cleft palate and (2) enabled researchers to compare TCDD-induced biochemical changes in palate tissue of several species. In vitro observations found that the human and rat palates are sensitive to the cleft palate formation through the same mechanisms seen in mice: changes in growth factors (i.e., EGF and TGFs) that are involved in the mechanism of altering programmed cell death in the medial epithelial cells of the palate. The response to TCDD in mouse palate cultures was about 100 to 1,000 times more sensitive than the response in human or rat palate cultures.

The available data provide substantial information to develop a qualitative model through which TCDD induces cleft palate. The induction of cleft palate in mice by TCDD is mediated through the Ah receptor. TCDD binds to the Ah receptor in the medial epithelial cells, and the activation of the Ah receptor initiates a cascade of events that increases TGF-beta 1 mRNA and protein, increases TGF-beta 2 and EGF receptor protein levels, and decreases TGF-alpha protein levels (Abbott et al., 1992). These changes alter the normal signaling pathways in the medial epithelial cells. In control animals, the interaction between these signaling pathways results in the programmed cell death of the peridermal medial epithelial cells. The alterations in growth factor regulation by TCDD result in continued proliferation of the peridermal medial epithelial cells and the redifferentiation of the basal epithelial cells to stratified squamous oral-like epithelial cells, which subsequentially prevents the fusion of the palate (Abbott and Birnbaum, 1989).

This preliminary model for the induction of cleft palate by TCDD requires better characterization of several steps. Structure-activity relationships indicate that the Ah receptor is involved, but there is limited evidence that the Ah receptor is present in the medial epithelial cells of the developing palate. Cytosolic fractions of embryonic palatal shelves do contain an Ah receptor, but which cells are expressing the Ah-receptor is undetermined (Dencker and Pratt, 1981). It is presently unknown if the increases in TGF-beta 1 mRNA is

mediated by the interaction of the Ah receptor with a DRE directly activating transcription of the TGF-beta 1 gene or if the increases in TGF-beta 1 mRNA are due to the initiation of a cascade of cytosolic or plasma membrane events mediated through the Ah receptor. Further research is indicated into the interaction of the growth factors and their specific role in palate formation. Because many of the data used to formulate this model are from studies using cultured palates, the development of quantitative models would require dose-response data for the in vivo alterations of these growth factors by TCDD, which are unavailable at this time.

Cleft palate in rats (Schwetz et al., 1973; Couture et al., 1989) and hamsters (Olson et al., 1990) is induced at doses that result in significant maternal toxicity and fetal mortality, and maximal induction of cleft palate is between 10% and 20%; however, in the mouse, cleft palate can reach 100% incidence before any fetal mortality or maternal toxicity is demonstrated. These data indicate that the mouse is extremely sensitive to this response. In vitro studies indicate that humans may be much less sensitive than mice to TCDD-mediated increases in cleft palate, so it is plausible that in humans cleft palate may occur only after high exposures.

8.2.6.2. *Hydronephrosis*

In mice, hydronephrosis is also produced by TCDD following prenatal exposure at doses that do not produce fetal mortality (Couture-Haws et al., 1991). Postnatal exposure prior to day 4 can also produce hydronephrosis in mice (Couture et al., 1989). The hydronephrosis induced by TCDD is due to occlusion of the ureter by epithelial cells (Abbott and Birnbaum, 1990b). Increased proliferation of the epithelial cells by TCDD is associated with increased EGF receptor. Hydronephrosis has not been reported in any other species at doses that do not result in significant fetal mortality (Birnbaum et al., 1991).

Mice are the only species in which TCDD produces frank terata at doses that are not fetotoxic. At present, there is no evidence that indicates humans are as sensitive as mice to these effects. The only available data comparing the sensitivity of fetal tissue demonstrate that human and rat fetal tissues are equally sensitive to the effects of TCDD (Birnbaum, 1991). These data suggest that sublethal exposure to TCDD may not result in frank terata of the kidney.

8.2.6.3. *Thymic Atrophy*

Prenatal exposure to TCDD produces thymic atrophy in all species tested and occurs at doses well below those that cause maternal or fetal toxicity (Birnbaum, 1991). Thymic atrophy occurs at similar doses in rats, guinea pigs, and hamsters exposed prenatally despite a 5,000-fold difference in the LD_{50} in the adult animals (Olson et al., 1990). The sensitivity and interspecies consistency of this response indicate that prenatal exposure to TCDD may result in thymic atrophy in humans. The mechanism of thymic atrophy has not been elucidated sufficiently to incorporate into a biologically based mechanistic model. Current research has focused on the TCDD-induced alterations in thymocyte development and their role in immunotoxicity.

8.2.7. Immunotoxicity

Although considerable research has focused on immunotoxicology of the dioxins (see Chapter 4, Immunotoxicity), we are not at present able to develop or test a biologically based model for purposes of risk assessment. A major obstacle to this undertaking is uncertainty as to the outcome to be modeled. Susceptibility to infection or impairment of graft versus host response could be proposed as the outcome for risk assessment, but not all studies have used these responses as end points. Moreover, this may not be a sensitive indicator of immune function. Alterations in biological markers of disease in animals or humans are not known. Some of the measurable biochemical responses of animals to TCDD may reflect successful response to xenobiotic challenge rather than impairment in functional integrity. Thus, changes in immunoglobulins may reflect immune competence rather than dysfunction. Our inability to define outcome is not unique to immunotoxicology; the continuing controversies over the definition of acquired immunodeficiency syndrome reflect scientific uncertainty in this area. NIEHS has proposed a tier approach to the identification of potential immunotoxicants (Luster et al., 1992), and dioxin certainly tests positive in this system.

Our knowledge of basic immunobiology makes it difficult for us to integrate our findings on dioxin into an overall biologically based schematic of events. We do not know the quantitative relationships between a change in intercellular signaling and cell-mediated

responses, although we know these events are fundamentally related. Many events in the immune system appear to have complex interactions, with biphasic relationships. Thus we have no quantitative context in which to develop predictive associations between events affected by dioxin and other events in immune system response.

We place high priority on improving our ability to develop risk assessment methods for immunotoxicants, not limited to dioxin. As noted above, progress has been made on developing a consensus approach to the hazard identification of potential immunotoxicants, but as yet there are no methods for using dose-response data from such tests to develop quantitative risk assessments. Dioxin may be a prototype compound for developing such methods, and research should be directed toward designs that encompass many different events in immunology from early molecular and cellular events to whole animal response to immune challenge, in order to facilitate the overall evaluation of end points. Moreover, in such designs, sufficient dose ranges should be used to assist in the statistical evaluation of proposed models and to compare animal and human responses.

In clinical and epidemiological studies, much data collection should be done; given the accessibility of circulating lymphocytes and other markers in blood, it should be possible to increase our confidence in interspecies comparisons by examining the same parameters in exposed animals and well-characterized human populations. Because of the reported sensitivity of the developing organism to immunotoxic effects of dioxin, a priority should be placed on obtaining data on immunologic function in children with documented exposures to dioxins or related compounds. Clinical studies need to be well controlled and conditions of testing and sample collection carefully described in order to facilitate such comparisons.

8.2.8. Reproductive Toxicity

8.2.8.1. *Female Reproductive Toxicity*

Several studies have demonstrated that TCDD affects female reproductive function in mice, rats, and monkeys. TCDD reduces fertility, litter size, and uterine weights. TCDD also alters menstrual and estrus cycling in monkeys, mice, and rats. Uterine weight and menstrual/estrus cycling are regulated by estrogens. These data indicate that TCDD has antiestrogenic effects that result in decreased reproductive functioning.

The antiestrogenic actions of TCDD could be mediated either by changes in circulating estradiol, qualitative changes in estrogen metabolism, or through decreases in estrogen receptors. In mice, TCDD does not alter serum estradiol levels, and the antiestrogenic actions of TCDD are associated with decreases in uterine estrogen receptors (DeVito et al., 1992). Similarly, TCDD decreases rat hepatic and uterine estrogen receptor (Romkes et al., 1987) but does not affect serum estradiol levels. Structure-activity studies suggest that the Ah receptor mediates the down-regulation of the estrogen receptor. The estrogen is down-regulated by TCDD in several breast cancer cell lines (Safe et al., 1992a). TCDD also decreases estrogen receptors in Hepa 1c1c7 cells but not in mutant cell types that do not express a high affinity form of the Ah receptor nor in cells that do not accumulate activated Ah receptors in their nucleus (Zacharewski et al., 1991). These studies provide further evidence that the Ah receptor is involved in the down-regulation of the estrogen receptor.

One possible mechanism for the antiestrogenic actions of TCDD is that TCDD binds to the Ah receptor in the target tissue and through a cascade of events decreases the amount of estrogen receptor in the cell, thus inhibiting the actions of estrogens. The down-regulation of the estrogen receptor by TCDD could be mediated either by decreased transcription of the estrogen receptor gene or possibly through nontranscriptional mechanisms. At present it is unclear how TCDD down-regulates the estrogen receptor other than it is mediated through the Ah receptor.

An alternative mechanism by which TCDD inhibits estrogenic actions is through increases in estradiol metabolism. Following TCDD exposure, estradiol metabolism is increased 100-fold in MCF-7 cells (Spink et al., 1990). Microsomal hydroxylation of estradiol is increased twofold to fourfold in rats treated with TCDD (Graham et al., 1988). The role of estrogen metabolism in the antiestrogenic actions of TCDD remains to be determined. While there is more evidence supporting the role for the down-regulation of the estrogen receptor mediating the antiestrogenic actions, further studies are required to determine the extent of estradiol metabolism in vivo following TCDD treatment.

Since TCDD alters immune function and a variety of growth factor pathways and in general is acting like a potent and persistent environmental hormone, research is needed to

determine the relationships, if any, to endocrine-related disorders in women such as endometriosis, osteoporosis, and cancers of the reproductive tract.

8.2.8.2. *Male Reproductive Toxicity*

When administered to adult rats, TCDD decreases testis and accessory sex organ weights, decreases spermatogenesis, and reduces fertility (Moore et al., 1985; Moore and Peterson, 1988; Bookstaff et al., 1990a). These effects are associated with decreases in plasma testosterone (Moore et al., 1985). The decreases in circulating androgens are due to decreased testicular responsiveness to luteinizing hormone and increased pituitary responsiveness to feedback inhibition by androgens (Moore et al., 1989, 1991; Bookstaff et al., 1990a, b; Kleeman et al., 1990). Although the antiandrogenic effects occur within 24 hours, the doses required to produce these effects are overtly toxic and decrease food intake and body weight. The high doses needed demonstrate that the antiandrogenic effects are not very sensitive effects. However, epidemiological studies have demonstrated decreased testosterone in workers exposed to dioxin-like compounds (Dioxin92).

In contrast to the adults, the developing male reproductive system is very sensitive to the effects of TCDD. In rats, prenatal exposure to TCDD results in decreases in sex organ weight, impairs spermatogenesis and luteinizing hormone secretion, and demasculinizes and feminizes sexual behavior (Mably et al., 1992a, b, c). Maternal doses as low as 0.064 μg/kg can produce these effects, indicating that the developing male reproductive system is one of the most sensitive end points for the toxic effects of TCDD. The alterations in male reproductive development are associated with decreases in testosterone levels. Sufficient testosterone levels are critical for sexual differentiation of the central nervous system; thus the decreases in testosterone levels may, in part, account for the TCDD-induced reproductive alterations in male rats. It is also possible that these effects are due in part to alterations in tissue sensitivity to testosterone (Mably et al., 1992c).

In summary, there is ample evidence that noncancer end points are extremely sensitive to the toxic effects of TCDD. The available data do not provide enough information to develop biologically based mechanistic models for all noncancer end points. For some of the noncancer effects of TCDD there is sufficient evidence for which proposed

mechanism may be modeled. Experimental evidence on cleft palate formation and male reproductive toxicity provides sufficient evidence to propose qualitative models that can be developed into mechanistic models. However, for immunotoxicity, thymic atrophy, neurobehavioral toxicity, and female reproductive toxicity, the mechanisms by which they occur are unknown and in some cases the target tissues remain undetermined. Furthermore, few if any of the molecular events beyond ligand binding to the Ah receptor are understood for these effects. The only information we have to develop mechanistic models is dose-response relationships. Future studies that better characterize target tissues and the molecular mechanisms underlying these events are indicated. Because of the importance of generating reliable estimates of the risk for noncancer effects, the development of biologically based dose-response models for these effects is an urgent research need.

8.3. COMPARATIVE END POINTS/QUALITATIVE COMPARISONS

There has been considerable discussion concerning the relative sensitivity of the several toxicological end points modified by exposure to TCDD. Many scientists and regulators have stated that carcinogenicity may not be the most sensitive end point. However, before one can compare the magnitude of these various effects, one must understand the limitations imposed by both statistics and biology on these types of relative potency comparisons. This section briefly outlines some of the problems with discussing relative potency across different end points. It provides no simple solutions to these problems. These solutions will take a combination of research (in both statistics and biology) and management decisions where research cannot yet be used to fill the knowledge gaps in the comparison.

There are two basic problems with the comparison of toxic potency across end points. These are (1) comparison of measures of mortality (or serious threats to mortality) to measures of morbidity or to measures of biochemical modifications and (2) statistical issues concerning the power to detect effects and the inclusion of background responses. We will discuss each of these in detail using TCDD as an example of the problems involved in this undertaking.

We will consider these issues in reverse order starting with the statistical problem first. Statements such as the one above sometimes reflect a statistical artifact due to the nature of the biological assay being analyzed. To illustrate this point, consider the data given in Table 8-4. This table considers four different responses to TCDD: liver cancer in female mice and rats, immunological changes in male mice, changes in protein concentrations in female rats, and rates of terata in mice. One practical way to address potency across end points is to rely on significant difference from control for each treatment group. On the basis of a statistical test, it is clear that the most sensitive end point is an effect on the immune system where a response was detected at a dose of 0.644 ng/kg. Moreover, the end point being measured (plaque-forming cell response) has a greater numerical value than do other responses (such as tumor incidence), with a small variability allowing for much greater statistical power in obtaining a response. Also, the lowest dose for induction of CYP1A1 shows a significant effect on immune responses; it is impossible to know if lower doses might not have been significant also. Thus the location of a no-observed-effect level (NOEL) is dependent on the statistical properties of the end point being studied and the sensitivity of a particular response.

One practical way would be to consider the relative change in response over background in the TCDD-treated groups. For example, the drop in plaque-forming cell (PFC) response from control to low dose is 6% or $\sim$0.06 relative units of response per 0.322 units (pg/kg/day) increase in dose, or a slope of 0.0186. This is $\sim$3 times greater than the relative increase in CYP1A1 (slope of 0.068) and about one-tenth the relative increase in liver tumors in female mice (National Toxicology Program, 1982). Even more dramatic is the infinite change in response from control (0.00) to low dose (0.019) for cleft palate in the Abbott and Birnbaum (1989) study. Thus, using this measure of relative potency, cleft palate is the most sensitive end point, with liver cancer second and the immunological response third. Thus, a second practical measure of potency, changes relative to background, paints a very different picture than does the use of statistical p-values and is sensitive to zero response in the control population. In addition, one has to scale between relative drops and relative increases, the one bounded by 0 and the other unbounded (although for practical purposes, this is easily dealt with). Finally, one could also use

Table 8-4. Toxic End Points Database

End Point	Dose[a]	Response	Reference
Liver cancer in female mice	0.0	0.041[c] (3/73)[d]	NTP (1982[b])
	1.4	0.120 (6/50)	
	7.1	0.125 (6/48)	
	71.0	0.234 (11/47)[e]	
Liver cancer in female rats	0.000	0.051[g] (3/58.4)[h]	Kociba et al., 1978[f]
	1.0	0.029 (1/34.0)	
	10.0	0.272 (9/33.1)[e]	
	100.0	0.607 (19/31/3)[e]	
Plaque-forming cells per 10^6 viable spleen cells	0.00	777±88	Davis and Safe, 1988[i]
	0.322	731±190	
	0.644	438±96[e]	
	1.191	118±46[e]	
	3.091	65±15[e]	
Concentration of cytochrome P450IA1 in microsomal protein form hepatocytes (pmol/mg)	0.0	12.9±11.3	Tritscher et al., 1992
	3.5	56.4±26.7[e]	
	10.7	111.5±30.3[e]	
	35.7	181.4±18.4[e]	
	125.0	293.3±17.1[e]	
Concentration of cytochrome P450IA2 in microsomal protein form hepatocytes (pmol/mg)	0.0	63.5±38.4	Tritscher et al., 1992
	3.5	88.3±23.0	
	10.7	161.0±55.7[e]	
	35.7	193.1±60.2[e]	
	125.0	297.4±88.3[e]	
Cleft palate in mice fetus	0.0	0.000 (0/159)	Abbott and Birnbaum, 1989
	6000.0	0.019 (2/107)	
	9000.0	0.213 (26/122)[e]	
	12000.0	0.505 (50/103)[e]	
	15000.9	0.777 (84/108[e]	

[a]In ng/kg/day
[b]Gavage dosing
[c]Probability of getting a tumor prior to the end of the study
[d]Number with tumor/number examined for the tumor
[e]Significantly different from control response ($p < 0.05$)
[f]TCDD in diet
[g]Survival-adjusted using poly-3 adjustment of Portier and Bailer (1989)
[h]Number with tumor/poly-3 adjusted number at risk of tumor
[i]Single intraperitoneal injection

absolute (rather than relative) change over background, but then one would be faced with the problem of determining how a change of one unit in PFC response relates to a change of one unit in CYP1A1 response.

The only practical manner in which a relative comparison of potency can be made is in terms of mortality. In this way, all responses are in the same units and have a common control response in terms of the background mortality in the population. However, this approach is currently infeasible. There are both technical and practical considerations that must be ironed out before an approach of this type can be applied. On the technical side, there are issues concerning life expectancy versus incidence of death. For example, suppose TCDD increased resorptions by 5% at some chosen dose. This would represent a 5% increase in mortality for potential fetuses and an overall loss of 5% of the total available number of animal lifetimes (resulting in a 5% drop in life expectancy). Suppose also that this same dose of TCDD increased mortality from cancer so that, by the end of the study, an additional 20% of the animals have died. Suppose also that this increase in mortality is late in life so that the overall drop in life expectancy is only 5%. Thus, on one scale (life expectancy), the two end points, resorptions and cancer, produce the same results, whereas on another scale, mortality by age, they are different. It is unclear which measure is most appropriate for ranking the observed effects of TCDD. On the more practical, biological side of the issue, one must relate increases in tumor incidence to changes in mortality, modifications in immune response and/or protein concentrations to changes in mortality, etc. The information needed to model these relationships is currently unavailable.

This is not to say that statements concerning the relative importance of certain end points for toxicity from exposure to TCDD cannot be addressed. However, caution must be used in this endeavor, and one must be careful to explain the methods by which the relative potencies were established. When this is done, the gaps in our knowledge become obvious and future research can be better directed to fill those gaps.

8.4. RELEVANCE OF ANIMAL DATA FOR ESTIMATING HUMAN RISKS

The reliability of using animal data to estimate human risks has been questioned, and this issue is especially important for TCDD. We know there are unusually wide species

differences in acute toxic responses to TCDD, but we do not know if such wide differences exist for carcinogenic and other toxic effects. However, we do know that the rank order of species differences in acute effects does not predict the rank order for all other toxic effects. For example, mice appear to be considerably more sensitive than rats to the teratogenic and immunotoxic effects of TCDD, but we do not know if dose-response relationships for immunotoxic effects in humans resemble those for rats, mice, or neither.

Although the human data are limited, it does appear that animal models are, in general, appropriate for estimating human risks, keeping in mind that for some responses, where wide species differences exist, the relative placement of human responses may not be possible at this time. However, humans contain a fully functional Ah receptor (Lorenzen and Okey, 1991; Manchester et al., 1987; Cook and Greenlee, 1989), and many of the biochemical effects produced by TCDD in animals also occur in humans. Data on effects of TCDD and its analogs in humans are based on in vitro (i.e., in culture) as well as epidemiological studies. A comparison of the effects of CDDs and CDFs on laboratory animals versus humans is given in Table 8-5. In vitro systems such as keratinocytes or thymocytes in culture have clearly shown that human cells possess Ah receptors, and they respond similarly to cells derived from rodents. Several reports in the literature suggest that exposure of humans to dioxin and related compounds may be associated with cancer at many different sites, including malignant lymphomas, soft tissue sarcomas, hepatobiliary tumors, hematopoietic tumors, thyroid tumors, and respiratory tract tumors (Bertazzi et al., 1989, 1993; Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990; Saracci et al., 1991). These studies are evaluated in Chapter 7, Epidemiology/Human Data, including discussion of confounding factors and strength of evidence. There is growing evidence from human cancer studies that TCDD is a multisite carcinogen, which is not unexpected if we assume that TCDD is acting like a potent and persistent hormone agonist/antagonist. Likewise, TCDD is a multisite carcinogen in animals (Lucier, Chapter 6).

Several noncarcinogenic effects of chlorodibenzodioxins (CDDs) and chlorodibenzofurans (CDFs) show good concordance between laboratory species and humans as well. For example, in laboratory animals, TCDD causes altered intermediary metabolism manifested by changes in lipid and glucose levels. Consistent with these results, workers

Table 8-5. Similarities Between Laboratory Animals and Humans in Biological Effects of TCDD

Effect	Laboratory animals	Human or human cells
In vitro		
Presence of Ah receptor	+	+
Enzyme induction	+	+
Altered pattern of growth and differentiation		+
Immunosuppression	+	+
Choracnogenic response	+	+
In vivo		
Presence of Ah receptor	+	+
Enzyme induction	+	+
Altered lipid metabolism	+	+
Immune effects	+	+/-
Cancer	+	+
Reproductive effects	+	+/-
Teratogenic effects	+	+/-
Altered epithelial cell differentiation	+	?
Tumor promotion	+	?

Source: Silbergeld and Gasiewicz, 1989

The + indicates a clear association while +/- indicates conflicting or unclear associations; the ? indicates that not enough is known on the effects of TCDD on the system to evaluate.

exposed to TCDD 7 to 8 years previously during the manufacture of trichlorophenol showed elevated total serum triacylglycerides and cholesterol with decreased high density lipoprotein (Walker and Martin, 1979). Recently, the results of a statistical analysis of serum dioxin analysis and health effects in Air Force personnel following exposure to Agent Orange were reported (Wolfe et al., 1990). Significant associations between serum dioxin levels and several lipid-related variables were found (percent body fat, cholesterol, triacylglycerols, and HDL). Another interesting finding of these studies was a positive relationship between dioxin exposure and diabetes, possibly the first report of such an association.

The human to experimental animal comparison is confounded by at least two factors: (1) For most toxic effects produced by dioxin, there is marked species variation. An outlier or highly susceptible species for one effect (i.e., guinea pigs for lethality or mice for teratogenicity) may not be an outlier for other responses. (2) Human toxicity testing is based on epidemiological data comparing "exposed" to "unexposed" individuals. However, the "unexposed" cohorts contain measurable amounts of background exposure to CDDs, CDFs, and dioxin-like PCBs. Also, the results of many epidemiological studies are hampered by small sample size, and in many cases the actual amounts of dioxin and related compounds in the human tissues were not examined. However, based on the available information, it appears that humans respond to CDDs and CDFs like most experimental animals.

There is also relatively good concordance in the biochemical/molecular effects of TCDD between laboratory animals and humans. Placentas from Taiwanese women exposed to rice oil contaminated with PCBs and CDFs have markedly elevated levels of CYP1A1 (Lucier et al., 1987). Comparison of these data with induction data in rat liver suggests that humans are at least as sensitive as rats to enzyme-inductive actions of TCDD and its structural analogs (Lucier, 1991). Consistent with this contention, the in vitro EC_{50} for TCDD-mediated induction of CYP1A1-dependent enzyme activities is ~ 1.5 nM when using either rodent or human lymphocytes (Clark et al., 1992). However, binding of TCDD to the Ah receptor occurs with a higher affinity in rat cellular preparations compared to humans (Lorenzen and Okey, 1991). This difference may be related to the greater lability of the human receptor during tissue preparation and cell fractionation procedures (Manchester et al., 1987). In any event, it does appear that humans contain a fully functional Ah receptor

(Cook and Greenlee, 1989) as evidenced by significant CYP1A1 induction in tissues from exposed humans, and this response occurs with similar sensitivity as observed in experimental animals.

One of the biochemical effects of TCDD that might have particular relevance to toxic effects is the loss of plasma membrane EGF receptor. There is evidence to indicate that TCDD and its structural analogs produce the same effects on the EGF receptor in human cells and tissues as observed in experimental animals. First, incubation of human keratinocytes with TCDD decreases plasma membrane EGF receptor, and this effect is associated with increased synthesis of TGF-α (Choi et al., 1991; Hudson et al., 1985). Second, placentas from humans exposed to rice oil contaminated with polychlorinated dibenzofurans exhibit markedly reduced EGF-stimulated autophosphorylation of the EGF receptor, and this effect occurred with similar sensitivity as observed in rats (Lucier, 1991; Sunahara et al., 1989). The magnitude of the effect on autophosphorylation was positively correlated with decreased birth weight of the offspring.

In summary, animal models are reasonable surrogates for estimating human risks. However, it must be kept in mind that the animal to human comparison would be strengthened by additional mechanistic information, especially the relevance of specific molecular/biochemical changes to toxic responses. It is also important to note that the mechanism of carcinogenesis (sequence of molecular events) may be quite different at different sites. For example, the mechanism responsible for TCDD-mediated lung cancer appears to be different from that responsible for liver cancer (see Chapter 6, Carcinogenicity of 2,3,7,8-TCDD in Animals).

8.5. HUMAN MODELS

8.5.1. Introduction

Unlike animal data where recent studies have allowed modeling for dosimetry, induced proteins, cell proliferation, and toxic effects, human data are very sparse. With regard to toxic effects, Chapter 7 presents recent evidence suggesting TCDD's effects on human reproduction, neurotoxicity, diabetes, and cancer. From a modeling viewpoint, male reproduction, diabetes, and thyroid cancer appear to be good candidates for modeling from a

"bottom-up" mechanistic approach, since TCDD's effects on male serum testosterone levels, the insulin receptor, and thyroid hormones have been documented. These, however, remain efforts for the future. The focus of this section will be on cancer, specifically liver cancer, respiratory cancer, and all cancers combined. There are two reasons for this. First, the emphasis on liver cancer in EPA's history of TCDD regulation demands it, and it is a logical sequence to Section 8.3. Second, the recent epidemiology evidence for respiratory cancer, soft tissue sarcoma, and all cancers combined suggests that dioxin is a human carcinogen.

Modeling for these cancers in humans, however, requires and receives different approaches than has been presented in earlier sections of this chapter. The approach used for liver cancer is one involving interspecies toxic equivalent liver doses, with extrapolation from a rat to human no-observed-added-effect level (NOAEL). The modeling approach used for the human epidemiology data for lung cancer and all cancers combined involves estimating human intake dose associated with cancer response and curve fitting both additive and multiplicative risk models to the data.

8.5.2. Modeling Toxic Effects in the Liver

One of the intrinsic features of PB-PK descriptions is that interspecies extrapolations can be attempted with reasonable confidence in the result, assuming that relevant changes are made in the configuration of the model to allow for changes in physiology, metabolism, and protein binding. The limiting factor with any modeling description is, however, the availability of relevant data sets. This is particularly true when attempting to model the pharmacokinetics of dioxins and dioxin-like chemicals in people. The use of a physiological pharmacokinetics description to analyze human TCDD exposure data was first attempted by Kissel and Robarge (1988). In this work the authors used a fugacity approach to examine the elimination of TCDD from humans using data derived from estimates of background exposure and tissue levels, half-lives from Ranch Hand veterans, and a self-exposure experiment by Poiger (Poiger and Schlatter, 1986).

This fugacity-based model attributes the distribution of TCDD as a simple partitioning process with expected dose-independent linear kinetics. In both rats and mice, as noted earlier, a disproportionate amount of TCDD is found in the liver with increasing dose. This

phenomenon cannot be described by simple solubility/partitioning alone. The dose-dependent liver-to-fat concentration ratio is a good indicator of this trend. In fact, analysis of data from people exposed to CDFs, congeners of TCDD, from consumption of contaminated oil shows a higher liver:fat ratio with increasing body burden (Carrier and Brodeur, 1991). More complex models need to be constructed to account for the nonlinearities in dioxin disposition.

Carrier and Brodeur (1991) constructed a toxicokinetic model for halogenated polycyclic aromatic hydrocarbons (HPAHs) in humans. This model is not a classical PB-PK model. The analysis begins with the observation that the tissue distribution of dioxin-like HPAHs in people and animals is body burden dependent. As the body burden increases (C_{body}), the proportion of that body burden associated with the liver (F_h) increases toward a maximum value (F_{max}). This has been previously described for rats (Abraham et al., 1988). The data on rats (Kociba et al., 1976), monkeys (McNulty et al., 1982), and people, using toxic equivalency factor conversions (Kuroki and Masuda, 1978), were examined using an empirical, Michaelis-Menton type saturable binding isotherm:

$$\text{Liver fraction } (F_h) = F_{max}\, C_{body} / (K_d + C_{body})$$

Considering the body burden as a surrogate for liver concentration, this equation can be loosely interpreted as the induction of binding species in the liver as dose increases. Indeed, analyses showed that the K_d was very similar for people and experimental animals, possibly indicative of similar protein-induction dynamics in various species. With different dioxin-like isomers F_{max} and K_d vary; this can be thought of as changes due to different binding affinities.

This empirical model fits the observed data in various species; however, it is a fitting exercise and not an examination of underlying biology. The model is not physiologically based. It in effect examines the steady-state condition of a two-compartment model, consisting of the liver and "the rest of the body." The terms C_{body} and F_{max} are difficult to interpret in biological terms. C_{body} represents a body burden of chemical rather than a tissue concentration, and the term for maximum liver concentration F_{max} is derived empirically.

In its present form the model assumes that at very low doses the hepatic fraction is zero. This is unlikely due to the partitioning of dioxin into the liver and the low-dose binding characteristics of the Ah receptor and CYP1A2 in the liver. Also in this description the metabolism is modeled as saturable at maximal induction of liver TCDD sequestration. Given the experimental data in many species (Chapter 1, Disposition and Pharmacokinetics), this seems unlikely. The analysis of human dioxin kinetics does show that the dose-response curves for and induction of hepatic-binding species for dioxin in rodents and people appear to be very similar. If one assumes that humans and rodents are also similarly sensitive in the toxic responses to TCDD, based on liver concentrations, some simple exposure calculations can be made. Interspecies comparisons of the daily intake of TCDD needed to reach equitoxic concentrations in the liver have been estimated by Carrier (1991). These are presented in Table 8-6. It is important to note that a number of assumptions are made to make these comparisons, and considerable uncertainty exists.

The integration of the toxicokinetic description put forward by Carrier, and the accompanying data sets, with the more physiological approaches described for rats by Andersen et al. (1993b) and Kohn et al. (1993) will provide an opportunity to further investigate the determinants of disposition of TCDD in humans.

The prediction for Carrier's model of 40 pg/kg-day for a no-effect dose for cancer is ~7,000 times as high as that currently used by EPA but only 6 to 7 times higher than that of other western countries (Kociba, 1991). However, this model assumes that humans are as sensitive as rats to a given tissue burden of dioxin, and it does not account for possible interindividual variation among humans. Also, it is specific for liver, so it does not predict cancer responses in other tissues such as lung or any other effects. It also does not attempt to predict biochemical effects such as enzyme induction, which occurs at much lower doses than 40 pg/kg/day (Vanden Heuvel et al., 1994).

8.5.3. Lung Cancer and All Cancers Combined

Data from four recent epidemiology retrospective cohort occupational studies provide evidence of the human carcinogenicity of dioxin. All showed increased mortality from respiratory cancer; the two largest (Saracci et al., 1991; Fingerhut et al., 1991) showed

Table 8-6. Rat and Human Comparison of Daily TCDD Intakes and Body and Liver Concentration for Equitoxic Response

Parameter	Low intake		High intake	
	Rat[a]	Human	Rat[b]	Human
Daily Intake (TCDD eq)	1 ng/kg	40.7 pg/kg	100 ng/kg	1 ng/kg
Total body concentration (TCDD eq/body)	61 ng/kg	70 ng/kg	1.45 μg/kg	1.2 μg/kg
Liver concentration (TCDD eq/kg)	540 ng/kg	540 ng/kg	24 $\mu g/kg^2$	24 $\mu g/kg^2$

Source: Carrier, 1991.
[a]Rat NOAEL (Kociba et al., 1978).
[b]Marked liver toxicity; tumors in rats.

increases in mortality from soft tissue sarcoma, and three (Fingerhut et al., 1991; Zober et al., 1990; Manz et al., 1991) showed increased mortality from all cancers combined. In the Fingerhut et al. (1991) study all three cancer type results showed significance only for the high-exposure, long latent period subcohort. The largest study, with 18,000 total workers (Saracci et al., 1991), showed no increase in overall cancer mortality, but those authors have not presented the data allowing for a latent period. Furthermore, the Saracci et al. (1991) study, unlike the other three, provides no way to quantitatively estimate TCDD exposure to their cohort. Based on this lack of information, this modeling exercise will be restricted to the other three cohorts.

Three other recent cohorts were not included in this analysis for various reasons. Kuratsune et al. (1988) reported increased lung cancer in male victims (standard mortality ratio [SMR]=3.3, based on eight cases) of the Yusho PCB and CDF contamination rice poisonings. Although there are serum measurements and 37 TEF estimates available for this cohort, there was no actual TCDD in the contaminants. Thus, this cohort also will not be used in the modeling effort here. Collins et al. (1993) reported increased mortality for both lung cancer and all cancers combined for a subcohort of 122 U.S. workers who developed chloracne following exposure to dioxin at a chemical plant during a 1949 accident. Their analysis, however, attributes this increase to co-exposure to 4-aminobiphenyl. Since that chemical plant is included in the Fingerhut et al. (1991) cohort, it will not be included in this analysis. The Seveso, Italy, community cohort is also not included in this analysis because of the limited observation period following the 1976 accident. All of these studies are discussed in much greater detail in Chapter 7, Epidemiology/Human Data.

The largest of the three studies used here is the Fingerhut et al. (1991) study of >5,000 U.S. workers from 12 U.S. plants producing chemicals contaminated with TCDD. Of 1,520 workers exposed to TCDD-contaminated processes for at least 1 year with a 20+ year latency, mortality was significantly increased for both respiratory cancer (SMR=142; 95% C.I. 103-192) and for all cancers combined (SMR=146; 95% C.I. 121-176). A similar-sized cohort with less than 1-year exposure with a 20+ year latency showed no increase in either all cancers or respiratory cancers. Manz et al. (1991), in a smaller cohort of 1,148 men in a herbicide manufacturing plant in Hamburg, Germany, also found increased

mortality from lung cancer (SMR=141; 95% C.I. 95-201) and all cancers combined (SMR=124; 95% C.I. 100-152). Cancer mortality increased both among groups with increased duration of exposure and among groups with suspected highest levels of exposure. In the smallest of these recent studies, Zober et al. (1990) studied three subcohorts totaling 250 workers with potential exposure to dioxin during an industrial accident in 1953. Of the 127 who developed either chloracne or erythema, and who were considered among the most highly exposed, for those with a 20+ year latent period, mortality from all cancers (SMR=201; 95% C.I. 122-315) and from lung cancer (SMR=252; 95% C.I. 99-530) was both statistically increased. Furthermore, the increase in total cancer deaths in all these studies does not appear to be due totally to the increase in respiratory deaths. The SMRs for all cancer deaths not including lung cancer remain statistically significant in all three studies.

These findings are supported by recent animal evidence from Lucier et al. (1991) who found lung tumors in ovariectomized female Sprague-Dawley rats but not in intact female rats following administration of TCDD. Increased lung tumors are also seen in the Kociba et al. (1978) study with female Sprague-Dawley rats but not with male rats. Other animal data support the tumor-promoting ability of dioxin in the liver and skin (Pitot et al., 1980).

Based on the evidence of lung cancer and all cancers combined, a quantitative analysis of dioxin's cancer potency will be modeled from the three epidemiology studies. All three studies attempted to verify dioxin levels in samples of their working cohorts, although in all cases the subjects were tested decades after exposure ended. Thus, with the limited information available, assumptions must be made about the representativeness of both these sampled subjects and the dose-response models used to estimate risk. The details are presented below.

8.5.3.1. *Dose-Response Models*

The following analysis provides maximum likelihood and 95% lower confidence limits of incremental cancer risk based on the cancer death response in the lung and all cancers combined in the three recent cohort studies (Fingerhut et al., 1991; Zober et al., 1990; Manz et al., 1991). Both additive and relative risk models are used. This type of analysis has been used previously with epidemiologic studies in several EPA health assessments (e.g.,

methylene chloride, nickel, and cadmium). For this report the analyses will be done both separately for each study and for all studies combined. A description of the models follows.

8.5.3.1.1. ***Excess or additive risk model.*** This model follows the assumption that the excess cause-age-specific death rate at age t due to dioxin exposure, $h_1(t)$, is increased in an additive way by an amount proportional to exposure at some age t-k. In mathematical terms, this is:

$$h_1(t) = \beta X_{t-k}$$

where X_{t-k} is the exposure at age t-k, and β, the parameter to be estimated, is the proportional increase. The total cause-age-specific rate h(t) is then additive to the background cause-age-specific rate $h_0(t)$ as follows:

$$h(t) = h_0(t) + h_1(t)$$

For an individual i observed from $t_{0,i}$ until age t_i, the cumulative death rate expected is:

$$H_i(t) = \sum_{t=t_{0,i}}^{t_i} S(t)h_0(t) + \beta \sum_{t=t_{0,i}}^{t_i} X_{t-k}$$

where S(t) = survival function to age t. For dioxin, with a long half-life, we can let k=0. For N_j individuals in exposure group J, the expected number of deaths is:

$$E_j = \sum_{i=1}^{N_j} H_i(t) = E_{0j} + \beta \sum_{i=1}^{N_j} \bar{X}_i \cdot W_i = E_{0j} + \beta X_j W_j$$

where E_j is the total number of expected cancer deaths in the observation period from the group exposed to average exposure X_j, E_{0j} is the expected number of cancer deaths due to background causes (lifetable "expected" rates), W_j is the number of person-years of observation for the jth exposure group, and the parameter β represents the slope of the dose-response model. To estimate β, the observed number of cause-specific deaths in group j, O_j, is assumed to be distributed as a Poisson random variable with expected value E_j. The

parameter estimate, b, can be tested for being significantly >0. A statistically significant result is evidence of an additional cancer effect due to dioxin exposure.

Under the above assumptions, the solution by maximum likelihood proceeds as follows: The likelihood equation is

$$L = \prod_{j=1}^{N} [\exp -(E_{0j} + \beta X_j W_j)] \; [E_{0j} + \beta X_j W_j]^{Oj} / o_j!$$

where N = the number of separate exposure groups. The maximum likelihood estimate (MLE) of the parameter β is obtained by taking the first derivative of the log likelihood equation, setting it equal to 0 and solving for b:

$$\frac{d \ln L}{d\beta} = \sum_{j=1}^{N} [-X_j W_j + (O_j X_j W_j / (E_{0j} + bX_j W_j))] = 0$$

The asymptotic variance for the parameter estimate b is:

$$[\sum_{j=1}^{N} X_j^2 W_j^2 / (E_{0j} + bX_j W_j)]^{-1}$$

where b is the MLE. This variance can then be used to obtain approximate 95% upper and lower bounds for β. Lifetime incremental cancer risk estimates for continuous exposure are estimated by multiplying b by 70 if X is in units of lifetime continuous exposure (i.e., lifetime average daily dose [LADD]).

8.5.3.1.2. ***Multiplicative or relative risk model.*** This model follows the assumption that the background cause-age-specific rate at any age t is increased in a multiplicative way by an amount proportional to the cumulative dose up to that age. In mathematical terms this is:

$$h(t) = h_0(t)(1 + \beta X_t)$$

As above, summing over the observed and expected experience yields, for each exposure group,

$$E_j/E_{0j} = 1 + \beta X_j$$

Again, to estimate β, the observed number of cause-specific deaths, O_j, assumed to be a Poisson random variable, is substituted for E_j. Following the same procedure as above, the MLE, b, is the solution to

$$\frac{d \ln L}{d\beta} = \sum_{j=1}^{N} -E_{0j}X_j + (O_j / (1 + bX_j)) = 0$$

with asymptotic variance

$$[\sum_{j=1}^{N} (E_{0j}X_j^2)/(1 + bX_j)]^{-1}$$

If X_j is in units of LADD, then lifetime incremental risk estimates per unit under this model are obtained by multiplying b by the background lifetime cause-specific risk of death, P_0. The values P_0 are derived using lifetable methods for competing risks and 1973-1977 U.S. death rates. For lung and all cancers combined, these are 0.038 and 0.185, respectively.

8.5.3.2. *Exposure and Dose Estimates*

Exposure estimates are derived from serum dioxin levels in workers sampled long after exposure ended and extrapolated backward using a first-order model for elimination (U.S. EPA, 1994) with a biological half-life of 7.1 years (Pirkle et al., 1989). In humans, dioxin deposits primarily in adipose tissues at normal exposure levels, although body deposition dose dependency has been shown in animal studies (see Chapter 2, Mechanism(s) of Action, and Section 8.5.2). In those rat studies, liver/fat concentration ratios increase with increasing dose, due to TCDD induction of the binding protein P4501A2 in the rat liver. However, although there is evidence that dioxin causes some human liver toxicity (Di Domenico and Zopponi, 1986) and that the dioxin-like PCB and dibenzofuran compounds can also cause liver cancer in humans (Kuratsune et al., 1988) (see Chapter 7), there is no direct evidence that TCDD induces cancer in human liver. Thus, although Table 8-6 presents rat-to-human liver concentration toxic equivalents for various rat exposures, this section uses

only human data. In humans, all estimates suggest that adipose tissue is the major storage compartment. Schlatter (1991) estimated a liver/fat concentration ratio of about 1/6. With a body adipose tissue fat weight of 15% to 20% and a liver weight of 2.5%, over 90% of stored dioxin will be in adipose tissue.

Direct initial exposure to the lung in these studies is also difficult to estimate. Both inhalation and skin absorption are the equally likely routes of initial exposure, but the exposure scenario cannot be distinguished. Di Domenico and Zapponi (1986) estimated that ~50% to 90% of TCDD exposure to the Seveso residents following the 1976 accident occurred via the dermal route, but they assumed 100% dermal and inhalation absorption. A more likely 1% to 10% dermal and 75% inhalation absorption estimate (U.S. Environmental Protection Agency, 1985) would project that the inhalation route provided the major TCDD exposure. To further complicate the situation, the cohorts discussed below are all occupational so that both dermal and inhalation exposure are highly likely.

The data on body concentration levels in the three studies are presented in Table 8-7. Fingerhut et al. (1991) measured serum levels adjusted for lipids in a sample of 253 of the workers from 2 of the 12 plants approximately 20 years after last known exposure. They found a highly statistically significant correlation ($r=0.72$; $p<0.0001$) between the logarithm of number of years of exposure to processes involving TCDD contamination and the logarithm of individual TCDD serum levels. Based on this correlation, they divided the sample into a high-exposure group (defined as those exposed more than 1 year) and a low-exposure group (those exposed <1 year). The mean TCDD level of the low-exposure group was 69 ppt, while that of the high exposure group was 418 ppt. Among the 176 sampled workers last exposed >20 years before, those with under 1 year of exposure ($n=81$) had a mean level of 78 ppt, and those with over 1 year of exposure ($n=95$) had a mean level of 462 ppt.

For the Zober et al. (1991) study, the serum-level data are based on a nonrandom sample of 28 survivors tested 32 years after the 1953 accident. These subjects were then classified into three groups by scenario of high (C1), medium (C2), or low (C3) chance of TCDD exposure, with the mean (median) levels of 60 (24.5), 25 (9.5), and 25 (8) ppt, respectively. An alternative breakdown (B) (see Table 8-7) by the 16 sample subjects who

Table 8-7. Measured Serum TCDD Levels and Estimated Levels at Time of Last Occupational Exposure to TCDD, Based on First-Order Elimination Kinetics and a Half-Life for Elimination of 7.1 Years

Study	Sample	Sample size	Concentration at test time (ppt)		Estimated[b] median concentration at time of last exposure adjusted for background (ppt)
			Including background	Median adjusted for background[a]	
Fingerhut et al., 1991	Surviving cohort of workers from Plants 1 and 2 tested approximately 20 years after last occupational exposure	253			Range 2,000-32,000[c]
	Exposed >1 year, all	119	Mean 418 Median 231	226	
	Exposed >1 year, ≥20-year latency	95	Mean 462	226[d]	1,770
	Exposed <1 year, all	134	Mean 69 Median 24	19	
	Exposed <1 year, ≥20-year latency	81	Mean 78	19[d]	150
Zober et al., 1991	Sample of survivors tested 32 years after the accident	28			
Breakdown A	High-exposure scenario	10	Mean 60 Median 24.5	19.5	450
	Medium-exposure scenario	7	Mean 25 Median 9.5	4.5	105
	Low-exposure scenario	11	Mean 25 Median 8.4	4.4	100
Breakdown B	Either chloracne or erythema	16	Mean 50 Median 15	10	230
	Neither chloracne nor erythema	12	Mean 25 Median 5.8	0.8	20

[a]Background adjustment by subtracting 5 ppt from median value.
[b]Estimation based on adjusted medians, since serum levels are not normally distributed.
[c]Calculated mean levels by Fingerhut et al. (1991) not adjusted for background.
[d]Estimated based on median for all exposed >1 year, since estimate not given for ≥20-year latency.

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Table 8-7 (continued)

Study	Sample	Sample size	Concentration		Estimated[b] median concentration at time of last exposure adjusted for background (ppt)
			Including background	Median adjusted for background[a]	
Manz et al., 1991	Sample of nonselected members of cohort tested approximately 31 years after 1954 TCP production stopped	48			
	High-exposure scenario	37	Mean 296 Median 137	132	2,750[e]
	Medium- and low-exposure scenario	11	Mean 83 Median 60	55	1,150[e]
Schecter et al., 1989	Sample of U.S. veterans of Operation Ranch Hand	10	Mean 22 Median 17 Range 7-55	12	250[f]
Schecter, 1991	Sample of U.S. general population	100	Mean 5.2	0	5.2[g]

[e]Assumes no TCDD exposure after 1954.
[f]Assumes no TCDD exposure after 1969.
[g]Continuous background exposure only, no adjustment.

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exhibited either chloracne or erythema versus those 12 who did not yields estimates of 50 (15) and 25 (5.8) ppt.

For the Manz et al. (1991) study, serum levels were measured in 48 unselected members of the cohort who had what the authors believed to be similar exposures to TCDD as the cohort. Workers were classified into three groups, having either high-, medium-, or low-exposure opportunities, and interviews with these 48 members led to a division of 37 into the high group (mean 296 ppt, median 137 ppt) and 11 into the medium- and low-exposure groups combined (mean 83 ppt, median 60 ppt).

Also included in Table 8-7 are measured serum levels of U.S. veterans of Operation Ranch Hand and a sample of 100 U.S. men from the general population. The mean U.S. estimate of 5 ppt is identical to that reported from four controls in the German population (Schecter et al., 1988). The Fingerhut et al. (1991) referent controls had a mean level of 7 ppt.

Table 8-7 also presents estimates of median TCDD concentrations at time of last exposure for the median levels of various cohorts, based on first-order elimination kinetics, assuming a 7.1 year half-life. To be consistent with the requirements of the model, background levels of 5 ppt are subtracted from each median before back extrapolation. The formula used is:

$$C_t = C_0 e^{-k_e t}$$

where C_t = concentration at time of measurement, C_0 = estimated concentration at time of last exposure, k_e = elimination constant (per year) = 0.098, and t = years since last exposure. For the Zober (1991) and Manz (1991) studies and the Fingerhut short-exposure subcohort, these concentrations C_0 can be considered to be from short-term exposure, and average fat concentrations can be calculated from the formula:

$$\bar{C} = \frac{1}{T}\int_0^T C_0 e^{-kt} dt$$

where T = length of study (see Table 8-8). For the Fingerhut long-exposure subcohort with an average exposure period of 6.8 years, average concentration during the exposure period is estimated as 50% of the calculated C_0 = 1770 ppt. Since the average length of followup for this subcohort is 30 years, the total time from start of exposure to end of exposure is estimated as 9 (30-21) years. This leads to a total concentration × time for this subcohort of (Table 8-8):

$$9 \times 850 + \int_0^{21} 1770 e^{-kt} dt = 24{,}750 \text{ ppt - years}$$

Table 8-8 presents calculations of equivalent exposure estimates to convert from the dose metric of total fat concentration × time to intake dose. The process is to (1) calculate the continuous lifetime average daily *uptake* dose that will produce an equivalent total concentration × time and (2) to estimate the *intake* (oral) lifetime average daily dose (LADD) that would result in the continuous *uptake* dose.

To calculate (1), the assumptions of steady state discussed in Chapter 6, Volume II of U.S. EPA (1994) appear appropriate. These lead to their eq. 6-11, which is:

$$D = \left[\ln \frac{2}{t_{\frac{1}{2}}}\right] V_f C_{f,ss}$$

where D = uptake dose, V_f = volume of distribution of fat, $C_{f,ss}$ = steady-state concentration of dioxin in fat, and $t_{1/2}$ = 7.1 × 365 = 2,591.5 days

To calculate D, set V_f = 14L and first calculate $C_{f,ss}$ from age 21 to the age at the end of study, which will yield the same average concentration for each of the subcohorts described. For the Fingerhut et al. long-exposure subcohort, the equivalent $C_{f,ss}$ is (24,750/42 years) = 589 ppt. Constant daily uptake, D, for this subcohort is then 30.2

Table 8-8. Estimates of Lifetime Average Daily Dose for Oral Intake Equivalence for TCDD Based on Total Concentration × Time Equivalence. Estimates of TCDD Concentration Adjusted for Background at Time of Sampling and Back-Calculated Using First Order Elimination Kinetics, by Cohort

Study	Estimated Median Fat Concentration at Time of Last Exp. Adjusted for Background[f] ppt	Year	Average Time from Start of Exposure to End of Study Years	Average Age at End of Study Years	Total Conc. × Time ppt - Years	Average Concentration From Start of Exposure to End of Study ppt	Equivalent Average Conc. for Exposure from Age 21 to Age at End of Study ppt	Constant Daily Uptake for Equivalent Average Conc. pg/kg-day	Lifetime Average Daily Dose for Oral Intake Equivalence pg/kg-day[e]
Fingerhut et al. (1991) ≥ 20-year latency									
Exposed >1 year	1770	(1966)	30[a]	63[c]	24,750	790	589	31.5	63.0
Exposed <1 year	150	(1966)	28[a]	56[c]	1,430	51	41	2.2	4.4
Zober et al., 1991									
Breakdown A									
High	450	(1953)	34[b]	70[d]	4,430	130	90	4.9	9.7
Medium	105	(1953)	34[b]	70[d]	1,030	30	21	1.1	2.3
Low	100	(1953)	34[b]	70[d]	980	29	20	1.1	2.2
Breakdown B									
Either chlorance or erythema	230	(1953)	34[b]	70[d]	2,260	67	46	2.5	5.0
Neither chloracne nor erythema	20	(1953)	34[b]	70[d]	200	6	4	0.2	0.4
Manz et al., 1991									
High-exp. scenario	2750	(1954)	37[b]	70[d]	27,300	738	557	30.0	60.0
Medium- and low-exp. scenario	1150	(1954)	37[b]	70[d]	11,422	309	233	12.5	25.0

[a] From Table 8-9. Person Years/Cohort Size + 20 years.
[b] Estimated from study descriptions.
[c] From Fingerhut et al., 1991.
[d] Estimated from study descriptions and comparison with Fingerhut et al.
[e] Assumes 50% absorption by oral route.
[f] From Table 8-7, dates in parentheses are estimated dates of last exposure.

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pg/kg-b.w. Daily intake, or calculation (2), assuming 50% absorption from dietary, is then estimated as 63 pg/kg-b.w.

8.5.3.3. *Calculation of Risk Estimates*

Table 8-9 presents the LADDs from Table 8-8, the estimated relative risks, and sample size information for the various subcohorts. Whenever the data could be found, the subcohorts with at least 20-year latency are presented, in order to coincide as closely as possible with the Fingerhut et al. (1991) cohort. For the Manz et al. (1991) study, no data on person-years at risk are available, so only the relative risk model could be used to estimate risk. For the other studies, both models could be used. The data are shown in Figure 8-11 and indicate trends with increasing LADDs for all three studies and for both respiratory cancers and all cancers combined.

U.S. EPA practice for presenting risk estimates based on human data has been to use point estimates or maximum likelihood estimates, rather than upper-limit risk estimates.

Calculations of the incremental unit risk estimates for lung cancer and all cancers combined are presented in Tables 8-10 and 8-11, respectively, for each of the three cohorts separately and all cohorts combined, for both the additive and multiplicative risk models. The results show statistically significant estimates of the slope parameter for the Fingerhut (1991) study, the Manz (1991) study, and all studies combined. Although the slope estimates for the Zober (1990) study are greater than those for the Fingerhut (1991) and Manz (1991) studies, the cohort is smaller and statistical significance is seen only for all cancer deaths combined in the subcohort with chloracne or erythema. Since the Fingerhut (1991) data provide the bulk of the weight, the estimates from the combined studies are closer to those based on the Fingerhut (1991) study alone than to the others.

Also shown in Tables 8-10 and 8-11 are estimates of the lifetime incremental cancer risk for 1 pk/kg-day LADD intake. These are derived by substituting the MLE estimates of B back into the age-specific hazard rates and deriving lifetime incremental risk estimates based on lifetable probabilities with competing risks (for practical purposes the procedure described in the table footnotes produces nearly the same results). For lung cancer these unit risk estimates range from $3x10^{-4}$ to $2x10^{-2}$ $(pg/kg\text{-}day)^{-1}$, with the estimates for all studies

Table 8-9. Estimated Lifetime Average Daily Doses and Relative Risks by Individual Study Cohort

Study	Cohort	LADD (pg/kg-day)	Cohort size N_i	Person years	Respiratory cancer deaths			All cancer deaths		
					Obs.	Exp.	Rel. Risk	Obs.	Exp.	Rel. Risk
Fingerhut et al. (1991)	Exposed >1 year all		5,172	116,748	96	84.5	1.13	265	229.9	1.15
	Exposed <1 year all									
	Exposed >1 year, ≥ 20-year latency	63.0	1,520	15,136	43	30.2	1.42	114	78.0	1.46
	Exposed <1 year, ≥ 20-year latency	4.4	1,516	12,299	19	18.4	1.03	48	46.8	1.02
Zober et al. (1990)	(≥20-year latency)									
	High-exposure scenario	9.7	57	673	3	1.25	2.52	7	4.20	1.67
	Medium-exposure scenario	2.3	74	563	2	1.03	1.94	8	3.37	2.38
	Low-exposure scenario	2.2	81	676	0	0.97	0	1	3.39	0.29
	Either chloracne or erythema	5.0	109	1,209	5	2.09	2.39	14	6.96	2.01
	Neither chloracne nor erythema	0.4	103	703	0	1.16	0	2	4.00	0.50
Manz et al. (1991)[a]	(≥20-year latency)[b]				N = 1,148			(Entry before 1955)		
	High-exposure exposure scenario	60.0	96[c]	No data	30	21.3	1.41	18	8.5	2.11
	Medium- and low-exposure scenario	25.0	200[c]	No data				33	23.1	1.43

[a]West German reference controls used for consistency with Fingerhut and Zober studies.
[b]Assumed because of entry before 1955; actual data unavailable.
[c]Entry before 1955.

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Figure 8-11. Relative Risks of Lung Cancer and All Cancer Mortality in Three Recent Studies of Workers Exposed to TCDD, by Estimated LADD Equivalence.

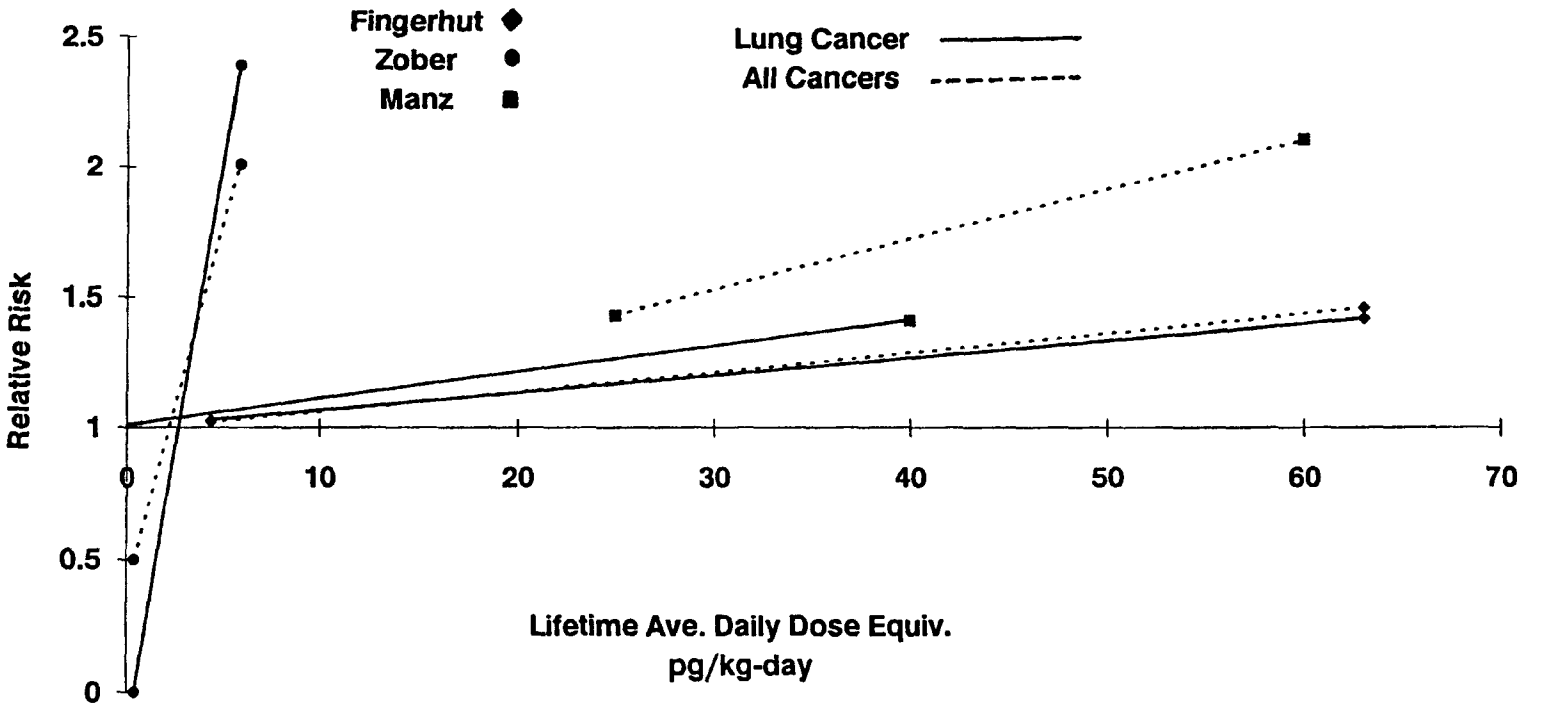

Figure 8-11. Relative risks of lung cancer and all cancer mortality in three recent cohort studies of workers exposed to TCDD by estimated LADD equivalence

Table 8-10. Calculation of Incremental Unit Cancer Risk Estimates and 95% Lower Limits for Both the Additive and Relative Risk Models Based on the Lung Cancer Deaths Response in the Fingerhut, Zober, and Manz Studies

Study	Model used	Parameter estimates b[a]	Asymptotic variance estimates	p-Value for slope[b]	Lifetime incremental cancer risk per 1 pg/kg-day LADD intake	
					Lower 95% limit	Maximum likelihood estimate[c]
Fingerhut et al., 1991	Additive	1.3×10^{-2}	4.69×10^{-5}	0.02	7.6×10^{-5}	4.7×10^{-4}
	Multiplicative	6.730	11.83	0.02	4.1×10^{-5}	2.6×10^{-4}
Zober et al., 1990	Additive					
	H-M-L scenario	2.18×10^{-1}	5.28×10^{-2}	0.17	0	7.6×10^{-3}
	Chloracne or not	4.45×10^{-1}	1.30×10^{-1}	0.11	0	1.6×10^{-2}
	Multiplicative					
	H-M-L scenario	126.55	16476.6	0.16	0	4.8×10^{-3}
	Chloracne or not	258.13	43519.2	0.11	0	9.8×10^{-3}
Manz et al., 1991[d]	Multiplicative	11.67	53.98	0.06	0	4.4×10^{-4}
All studies combined	Additive[e]	1.38×10^{-2}	4.73×10^{-5}	0.02	8.8×10^{-5}	4.8×10^{-4}
	Multiplicative[f]	7.862	9.90	0.01	1.0×10^{-4}	3.0×10^{-4}

[a]Estimates for additive risk model given in $(ng/kg\text{-}day)^{-1}$. Estimates for multiplicative risk model given in $(ng/kg\text{-}day)^{-1}/h_0(t)$ where $h_0(t)$ = background age-specific hazard rate.
[b]One-sided test based on asymptotic variance.
[c]MLE for additive risk model approximated by multiplying b by 35 since risk before age 35 is close to zero. MLE for multiplicative risk model approximated by multiplying b by $P_0=0.038$.
[d]Dataset in Table 8-9 with estimated LADD = 40 pg/kg-day.
[e]Fingerhut and Zober (chloracne) cohorts only.
[f]Fingherhut, Zober (chloracne), and Manz. See also footnote d for Manz.

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Table 8-11. Calculation of Incremental Unit Risk Estimates and 95% Lower Limits for Both the Additive and Relative Risk Models Based on the Total Cancer Deaths Response in the Fingerhut, Zober, and Manz Studies

Study	Model used	Parameter estimates b[a]	Asymptotic variance estimates	p-Value for slope estimate[b]	Lifetime incremental cancer risk per 1 pg/kg-day LADD intake	
					Lower 95% limit	Maximum likelihood estimate[c]
Fingerhut et al., 1991	Additive	3.76×10^{-2}	1.24×10^{-4}	0.0004	1.4×10^{-3}	2.6×10^{-3}
	Multiplicative	7.32	4.70	0.0004	7.0×10^{-4}	1.4×10^{-3}
Zober et al., 1990	Additive					
	H-M-L scenario	4.62×10^{-1}	1.46×10^{-1}	0.11	0	3.2×10^{-2}
	Chloracne or not	1.11	0.37121	0.03	8.1×10^{-3}	7.8×10^{-2}
	Multiplicative					
	H-M-L scenario	78.72	3950.1	0.11	0	1.5×10^{-2}
	Chloracne or not	192.62	11207.05	0.03	3.9×10^{-3}	3.6×10^{-2}
Manz et al., 1991[d]	Multiplicative	18.03	40.57	0.002	1.4×10^{-3}	3.3×10^{-3}
All studies combined	Additive[e]	3.83×10^{-2}	1.25×10^{-4}	<0.001	1.4×10^{-3}	2.7×10^{-3}
	Multiplicative[f]	8.93	4.33	<0.0001	1.0×10^{-3}	1.7×10^{-3}

[a]Estimates for additive risk model given in $(ng/kg\text{-}day^{-1})$; estimates for multiplicative risk model given in $(ng/kg\text{-}day)^{-1}/h_0(t)$ where $h_0(t)$ = background age-specific hazard rate.
[b]One sided, based on asymptotic variance; likelihood ratio tests generally gave smaller p-values.
[c]MLE for additive risk model approximated by multiplying b by 70; MLE for multiplicative risk model approximated by multiplying b by P_0=0.185.
[d]See footnote d, Table 8-10.
[e]Fingerhut and Zober (chloracne) cohorts only.
[f]Fingerhut, Zober (chloracne), and Manz.

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combined between $3x10^{-4}$ and $5x10^{-4}$ $(pg/kg\text{-}day)^{-1}$. For all cancers combined the range of MLE estimates is between $1x10^{-3}$ and $8x10^{-2}$ $(pg/kg\text{-}day)^{-1}$ with the estimates based on all studies combined between $2x10^{-3}$ and $3x10^{-3}$ $(pg/kg\text{-}day)^{-1}$.

These estimates from all studies combined (both lung cancer and total cancers) range from $3x10^{-4}$ to $3x10^{-3}$ $(pg/kg\text{-}day)^{-1}$. They exceed the upper-limit estimate of $1.6x10^{-4}$ $(pg/kg\text{-}day)^{-1}$ previously derived by EPA (U.S. EPA, 1985) based on the total cancer response in the female Sprague-Dawley rat in the Kociba et al. (1978) lifetime feeding study and the LMS model. Using the same Kociba (1978) study and LMS model but with the liver histopathology rereadings from a recent reanalysis (Sauer and Goodman, 1992) and original Kociba readings for the other tumor sites, the upper-limit estimate is $0.8x10^{-4}$ $(pg/kg\text{-}day)^{-1}$. Based on the LMS model and only the liver tumors (Sauer and Goodman reanalysis), the upper-limit estimate is $0.5x10^{-4}$ $(pg/kg\text{-}day)^{-1}$. This compares closely with the MLE estimate (for rats) of 0.24 $(pg/kg\text{-}day)^{-1}$ provided by the two-stage model in Section 8.2.2, which uses the same liver tumor pathology, together with additional liver foci data. Using a default $(body\text{-}weight)^{¾}$ power for rat-to-human conversion, the two-stage MLE estimate becomes 0.9 $(pg/kg\text{-}day)^{-1}$. These estimates are shown in Table 8-12.

8.5.3.4. *Low-Dose Deviation From Linearity*

Based on the LADDs and the relative risk estimates presented in Table 8-9, some idea of the degree of nonlinearity in the dose response for these cancers can be derived. For the Fingerhut et al. (1991) cohort, the ratio of 14.3 for high to low LADDs (63.0/4.4) corresponds to a ratio of increased risk of 14 (0.42/0.03) for respiratory cancer and 23 (0.46/0.02) for all cancer mortality combined. For the Manz et al. (1991) cohort, the comparisons are also consistent with linearity; the LADD ratio of 2.4 (60/25) corresponds to an increased risk ratio of 2.6 (1.11/0.43). The Zober et al. (1990) chloracne versus no-chloracne cohort with a high-to-low dose ratio of 12 suggests some low-dose sublinearity, but the low-dose estimate is very close to background and no quantitative comparisons can be derived, since the relative risk estimates for this low-dose group are < 1. For the Saracci et al. (1991) cohort, no direct comparison can be made either, except to note that the relative risk for lung cancer for the low-exposure group was actually higher than that for the high-

Table 8-12. Estimates of U.S. EPA Unit Cancer Risk for TCDD Oral Intake, Based on Animal and Human Studies and U.S. EPA Current and Proposed Estimates

Source	Cancers	Oral Dose Range	Model	Estimates of Unit Risk $(pg/kg\text{-}day)^{-1}$ MLE	95% Upper Limit	Comments	Ref. for Calculation
Animal (Female Sprague-Dawley rat)	Liver						
All Based on Kociba et al. (1978)		1-100 ng/kg-day	2-Stage[a]	0.9×10^{-4a}	—	Liver Pathology Readings by Sauer and Goodman (1992)	Chapter 8, Section 8.2.2
			LMS	—	0.5×10^{-4}		U.S. EPA (1992)[b]
			LMS	—	0.8×10^{-4}		U.S. EPA (1992)[b]
	All (liver, lung, hard palate/nasal turbinate)		LMS	1.2×10^{-4}	1.6×10^{-4} [d]	Liver Pathology by Kociba (1978) and Squire (1980)	U.S. EPA (1985)
			Multistage Weibull (Incidental Tumor Analysis)	2.1×10^{-4}	3.1×10^{-4}		U.S. EPA (1988)
Human (Males)							
Fingerhut et al. (1991)	Lung	1-60 pg/kg-day[c]	Additive Risk	4.8×10^{-4}	•	Calculations based on combined cohorts	Chapter 8, Section 8.5.3.3
Zober et al. (1990)	All		Multiplicative Risk	3.0×10^{-4}	•		
Manz et al. (1991)			Additive Risk	27×10^{-4}	•		
			Multiplicative Risk	17×10^{-4}	•		
U.S. EPA Proposed (1988)			Based on reciprocal of risk specific dose for 10^{-6} incremental risk	0.1×10^{-4}		External Review Drafts	U.S. EPA (1988)
U.S. EPA Currently Proposed (1994)				1×10^{-4}			Chapter 9, Risk Characterization

[a]Animal estimate of 0.24×10^{-4} $(pg/kg\text{-}day)^{-1}$ times rat-to-human default conversion of $(70/0.350)^{0.25}$.
[b]Unpublished.
[c]Estimates based on total concentration x time equivalence.
[d]Estimate currently used by U.S. EPA.
[e]EPA practice is to use MLEs for estimates based on human data.

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exposure group. Thus, for two of the cohorts there is some suggestion of sublinearity in dose response, but for the other two linearity is appropriate.

Estimates of LADDs of TCDD intake in the general U.S. population range from 0.3 to 1.0 pg/kg-day (U.S. EPA, 1994). The LADDs estimated in three epidemiology studies analyzed range from 0.4 to 63 pg/kg-day above background, with increased risks suggested in the 2-5 pg/kg-day. The LADD estimates themselves are just too imprecise for more definitive statements (see discussion in Section 8.5.3.5 below).

8.5.3.5. *Uncertainties in Estimates From Human Epidemiology*

There are many uncertainties associated with the unit risk estimates just derived from the epidemiology studies, the two largest being hazard identification and dose estimation. The evidence for a dioxin lung cancer hazard in humans is suggestive but not conclusive (see Chapter 7), while that for all cancers combined has less certainty. The estimates of dose, while based on actual body measurements, may lack both representativeness and precision. Although 253 subjects were sampled in the Fingerhut study, they were all taken decades after last exposure and were from two plants. Subjects from the larger plant, plant 1, had the higher dioxin levels but a lung cancer SMR=72 based on seven deaths, while the smaller plant had only one death from lung cancer (SMR=155). Analysis by plant in the Fingerhut study would have been possible if body measurements at these other 10 plants had been available.

Two choices of parameters, both of which affect LADD estimates by approximately a factor of two, provide some estimate of uncertainty. First, for back calculation for estimates of total body burden, a one-compartment first-order elimination model with a human half-life of 7.1 years has been assumed. Recent data, however, suggest a longer half-life of 11.3 years (Wolf et al., 1994). Use of this longer half-life would increase the unit risk estimate by about 40%. On the other hand, the body levels measured were quite variable and not symmetrically distributed within each study. This led to the selection of a median rather than a mean fat concentration for back extrapolation of the dioxin levels. If the mean had been used, the unit risk estimates based on these studies would have been approximately 50% to 70% less and much closer to those from the animal studies. Also, the estimated exposure in

the Zober chloracne subcohort is lower than levels thought to cause chloracne. This could explain why the unit risk estimates based on the Zober study are higher than those of the other two.

Another uncertainty is that of a possible interaction between dioxin and tobacco smoking. In mice, dioxin and 3-methylcholanthrene (3-MC, one of the many polyaromatic hydrocarbons in tobacco smoke) have been shown to be cocarcinogenic (Kouri et al., 1978; U.S. EPA, 1985). Other studies of mouse skin tumors have shown that dioxin can have anticarcinogenic properties when administered before initiation with either 3-MC or benzo(a)pyrene (U.S. EPA, 1985). Furthermore, dioxin's tumor-promoting ability suggests that two-stage models would be more appropriate if individual smoking histories were known. Individual smoking histories are presented only for the 37 cancer cases and deaths in the Zober cohort; only 2 were stated as being nonsmokers. All seven men with lung or larynx cancer were smokers. While characteristics of the two subcohorts in Fingerhut et al. (1991) suggest similar smoking prevalence, the effects with higher levels of dioxin could be synergistic.

A synergism of dioxin with 4-aminobiphenyl, a known human bladder carcinogen, has been suggested (Collins et al., 1993) for one of the plants in the Fingerhut study, but tobacco smoking would be present in all the plants and would seem to be a more likely universal effect modifier.

8.5.3.6. *Conclusions*

Epidemiology studies suggest that the lung in the human male is a much more sensitive target organ for TCDD than is the liver and that the human is a sensitive species for cancer response, probably more sensitive than is the rat. Although smoking may be a modifier for the lung cancer response, the studies also show increases for all cancers combined. Estimates derived from the human data suggest a unit risk for lung cancer of 3 to 5×10^{-4} $(pg/kg\text{-}day)^{-1}$; for all cancers combined the unit risk estimate is 2 to 3×10^{-3} $(pg/kg\text{-}day)^{-1}$. While unit risk estimates based on rat tumors are somewhat less, they are within the range of uncertainty of those based on human data. Both animal and human responses are consistent with low-dose linearity.

8.6. KNOWLEDGE GAPS

Considerable information is now available on the mechanisms of action responsible for TCDD's effects in experimental animals and humans, and important new information is now being generated. These data are, of course, essential to the development of reliable biologically based models for the estimation of human risks as a consequence of exposure to TCDD and its structural analogs such as the CDFs and coplanar PCBs. Uncertainty in such models reflects incomplete knowledge of mechanisms and inadequacies in exposure/tissue dose relationships. In the process of developing and evaluating biologically based models, we can identify those knowledge gaps that create uncertainty. The idea that interaction of TCDD with the Ah receptor is an essential first step in most, if not all, of dioxin's effects has been considered as a reasonable assumption for over a decade. The recent Banbury Conference on dioxin formalized this as a general consensus among dioxin researchers. The development of models that accurately predict risks also requires tissue and cell dosimetry data in experimental animals and humans. This kind of dosimetry information is available for blood, liver, and adipose tissue, but dosimetry data in other target tissues such as the lung, skin, pituitary, and reproductive tract are not available or incomplete. It would be especially relevant to the development of biologically based dose-response models to have dosimetry data (relationship between exposure, dose, and cell-specific dose) in target cells when the target cell is known. For example, the lung is composed of numerous cell types, but the identity of the target cell(s) for TCDD-mediated lung cancer is not known nor are there many data on dose-response relationships for concentrations of TCDD in whole lung or discrete cell types. Since the vast majority of dioxin is found in the liver and adipose tissue under chronic exposure steady-state conditions and the lung is clearly a target organ for biochemical and toxic effects, it would seem that the lung and perhaps other organs require far less tissue/cell levels of dioxin to exhibit toxic effects than the liver.

One of the most confounding yet important knowledge gaps in the development of mechanistic models is the evaluation of the adverse health consequences, if any, of current background exposure to the CDDs and CDFs, which is estimated at 1 to 3 pg TCDD equivalents/kg/day. More accurate information on the potency of dioxin-like PCBs is also an

essential component in evaluating the health impact of background exposure to chemicals that bind the Ah receptor.

Many of the molecular events that follow binding of TCDD to the Ah receptor are now known for transcriptional activation of the CYP1A1 gene. However, there is little information on the characterization of analogous events for dioxin's many other effects on gene expression such as Ah receptor-mediated alterations in the EGF or estrogen receptor. Most of the mechanistic or dose-response information on dioxin's effects has been generated on changes in gene expression of single genes such as CYP1A1 induction. There is only limited information on the complex interaction of biochemical, molecular, and biological events that are necessary to produce a frank toxic effect such as cancer, developmental defects, reproductive effect, or neurological effects. Figure 8-12 summarizes the series of interconnected steps within the three major components of receptor-mediated events (recognition, transduction, and response). Although this scheme is simplified (i.e., each step may comprise several events), it does provide a framework for identifying knowledge gaps that create uncertainty. Clearly, interactions with other endocrine systems are involved in some effects, and our ability to construct accurate dose-response models for noncancer end points would be enhanced if we had a better understanding of TCDD/endocrine interactions.

One of the more active areas of research on hormone action is directed at identifying the cell-specific factors that produce diversity of responses for receptor-mediated responses, that is, how do a single receptor and a single ligand produce the wide spectrum of cell-specific responses characteristic of exposure to a given hormone. Since TCDD is acting like a potent and persistent hormone agonist/antagonist, the mechanisms responsible for qualitative and quantitative differences in dose-response relationships for Ah receptor-mediated events might be similar to those mechanisms identified for steroid hormones. Fuller (1991) has summarized some of the mechanisms responsible for generating diversity, and these are listed below:

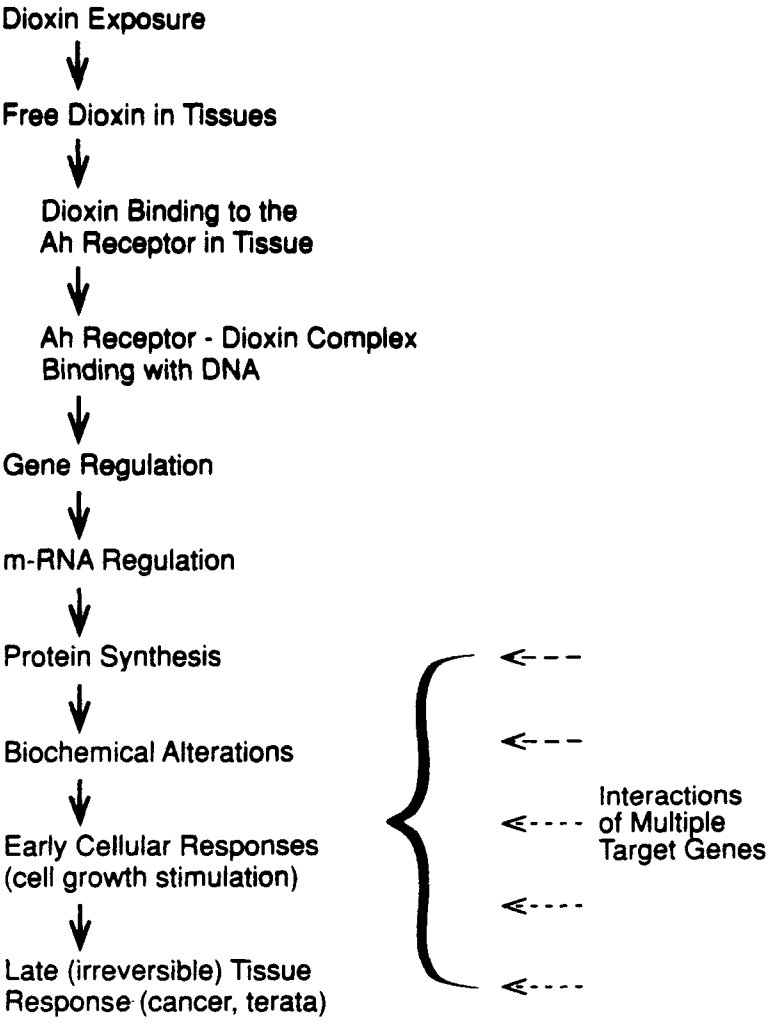

Figure 8-12. Biologically based risk assessment approaches for dioxin: Filling the gaps

ligand < ——————————agonist, antagonist

target tissue < ——————————receptor gene expression
activating or inactivating enzymes
binding proteins (extra- or intracellular)

receptor < ——————————cytoplasmic versus nuclear
isoforms-differential
splicing
gene duplication

dimers < ——————————hetero- or homodimers
DNA binding factors

nuclear factors < ——————————antagonist isoforms
squelching

response elements < ——————————consensus versus nonconsensus
number of copies
position
proximity of other response elements

transactivation < ——————————gene-specific factors
cell-specific factors

In addition to the above considerations, there is considerable speculation regarding the normal cellular functions of the Ah receptor and the identity of any endogenous ligands for the Ah receptor. If sound scientific information were available on the normal functions of the receptor, especially if those functions involve regulation of cell proliferation and differentiation, it would greatly enhance our ability to predict the health consequences of low-level dioxin exposure. It would also help considerably in the selection of appropriate animal models for estimating dioxin risks.

Interindividual variation in human responses to TCDD and its structural analogs is one of the most difficult issues to accommodate in the development of biologically based dose-response models. We know from epidemiology studies that some individuals develop

chloracne from a given exposure to dioxin, whereas other individuals exposed to the same amount of dioxin do not develop chloracne. The mechanisms responsible for sensitivity or resistance to the chloracnegenic actions of dioxin are not known, nor is there any information on the relationship of chloracne to other toxic effects. For example, are individuals who are susceptible to chloracne also susceptible to the carcinogenic actions of dioxin? Likewise, there are considerable differences in the magnitude of enzyme induction when human cells are cultured with dioxin. We need to understand the molecular mechanisms responsible for these differences and whether high inducers are more or less susceptible to the toxic effects of dioxin and its structural analogs. These kinds of data would allow the development of epidemiologic and laboratory approaches for evaluating health consequences in both sensitive or resistant populations.

In summary, we have gained considerable and valuable insights regarding mechanisms of dioxin and dose-response relationships for dioxin effects. These data are not yet complete but are appropriate for the development of preliminary biologically based models that may eventually be useful for estimating dioxin's risks to humans. When sufficiently developed, these models should provide increased confidence and decreased uncertainty than are present with the current default approaches (LMS or safety factor). They should also accommodate new scientific information from research directed at filling knowledge gaps to further reduce uncertainty. Based on the model structures presented in this chapter, it should be possible to design specific experiments to fill key knowledge gaps.

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APPENDIX A

A Mechanistic Model of Effects of Dioxin on Gene Expression in the Rat Liver

MICHAEL C. KOHN, GEORGE W. LUCIER, GEORGE C. CLARK, CHARLES SEWALL, ANGELIKA M. TRITSCHER, AND CHRISTOPHER J. PORTIER

National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

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Improved methods for estimating the shape of the response curve for effects of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are needed in order to evaluate possible adverse health effects of TCDD. A mathematical model has been constructed to describe TCDD-mediated alterations in hepatic proteins in the rat. In this model it was assumed that TCDD mediates increases in the liver concentration of transforming growth factor-α (TGF-α) by a mechanism which requires the aryl hydrocarbon (Ah) receptor. TGF-α subsequently binds to the epidermal growth factor (EGF) receptor, a process which is known to cause internalization of this receptor in hepatocytes. This action is thought to be an early event in the generation of a mitogenic signal. Because TCDD decreases binding of EGF in the livers of intact female rats but not in ovariectomized rats, this effect was further assumed to be dependent on estrogen action. The model postulates Ah receptor-dependent effects on the concentration of cytochrome P450 1A2 (CYP1A2), which is involved in the metabolism of estradiol, and on the concentration of the estrogen receptor. The model also incorporates information on induction of cytochrome P450 1A1 (CYP1A1) by TCDD. The biochemical response curves for all these proteins were hyperbolic (Hill exponents in the equations for their expression were found to be 1), indicating a proportional relationship between target tissue dose and protein concentration at low administered doses of TCDD. The model successfully reproduced the observed tissue distribution of TCDD, the concentrations of CYP1A1 and CYP1A2, and the effects of TCDD on the Ah, estrogen, and EGF receptors over a wide dose range. © 1993 Academic Press, Inc.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent carcinogen which is associated with increased incidence of liver tumors in female rats but not in male rats (Kociba *et al.*, 1978; National Toxicology Program, 1982; Clark *et al.*, 1991). Similarly, TCDD enhances hepatocyte proliferation and stimulates development of enzyme-altered hyperplastic foci in intact female rats but not in ovariectomized rats (Lucier *et al.*, 1991), suggesting that estrogens play a major role in TCDD-mediated hepatocarcinogenesis.

Biochemical signals regulating cellular proliferation are mediated in many tissues, including the liver, by the epidermal growth factor (EGF) receptor (Schlessinger *et al.*, 1983). The EGF receptor is a member of a family of plasma membrane receptors that, on binding of ligand, transduce signals by tyrosine kinase activity and by internalization of the liganded receptor (Hunter and Cooper, 1985). Phosphorylation of various proteins by this tyrosine kinase leads to alterations in cellular regulation and mitotic activity.

Livers of TCDD-treated rats show a dose-dependent decline in the maximal binding of EGF (Lucier, 1991) although TCDD has no effect on the amount of mRNA for the EGF receptor in mouse liver (Lin *et al.*, 1991a). The effect of TCDD on mRNA for the EGF receptor is a tissue-specific response. TCDD affects the amount of mRNA for the EGF receptor in the uterus (Astroff *et al.*, 1990) but not in keratinocytes (Osborne *et al.*, 1988). Antibodies raised against the EGF receptor stain the plasma membranes of hepatocytes in control rats, but in TCDD-treated rats there is an apparent redistribution of the receptor into the cytosol (C. Sewall and A. M. Tritscher, unpublished results). This is consistent with the notion that the loss of EGF binding capacity in liver plasma membranes is due to internalization of the liganded receptor.

The liver does not produce EGF, but there is evidence that it does produce several other EGF-like peptides such as transforming growth factor-α (TGF-α), another ligand of the EGF receptor (Mead and Fausto, 1989). TCDD induces expression of TGF-α in keratinocytes (Choi *et al.*, 1991), suggesting that TCDD may also induce TGF-α in the liver. Increased production of this peptide would subsequently stimulate EGF receptor-mediated events in that organ.

In order to obtain a quantitative relationship between exposure to TCDD and consequent alterations in the properties of the EGF receptor, we have constructed a mathematical model of the tissue distribution of TCDD in the rat

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and its effect on the concentrations of several important liver proteins. The model includes equations for the aryl hydrocarbon (Ah) receptor-dependent induction of cytochrome P450 isozymes 1A1 (CYP1A1) and 1A2 (CYP1A2) and of the Ah receptor itself. The complex between this receptor and TCDD (Ah–TCDD) is treated as inducing expression of TGF-α although the induced ligand of the EGF receptor actually may be another EGF-like peptide. It is also assumed that estrogen action is required for TCDD-mediated induction of TGF-α. TGF-α is modeled as released into the liver interstitium. From there it binds to the EGF receptor, causing its internalization. The model calculates the distribution of the receptor between the plasma membrane and cytosol.

Because estrogens appear to be required for TCDD-mediated effects on the EGF receptor, production of the estrogen receptor, CYP1A2-catalyzed formation of catechol estrogens, and deactivation of estrogens by glucuronidation were included in the model. In this effort, data on the stimulation of estrogen receptor synthesis by estradiol and the inhibition of estrogen receptor synthesis by TCDD were incorporated.

The model's predictions were compared to the data of Tritscher *et al.* (1992) and Sewall *et al.* (1993). Their experiments were a two-stage protocol in which female Sprague–Dawley rats were injected with an initiating dose of diethylnitrosamine (DEN). After 14 days the rats were subjected to biweekly gavage with TCDD in corn oil at doses equivalent to 3.5–125 ng/kg/day for 30 weeks. The rats were killed 1 week following the last dose, and their livers were assayed for TCDD, CYP1A1, CYP1A2, and plasma membrane binding of EGF. All such measurements were performed using samples from the same livers.

This model was used to predict tissue concentrations of TCDD and concentrations of induced proteins following administration of TCDD. A model which reproduces the dose–response relationships of experimental data and is consistent with the biochemical and physiological processes known to occur in rats exposed to TCDD might permit extrapolation of responses beyond the range obtained from experimental data and lead to scientifically sound approaches for estimating risks of adverse health effects of exposure to TCDD.

METHODS

The present model, hereafter referred to as the NIEHS model (see Appendices 1 and 2 for a complete description), was adapted from the physiologically based pharmacokinetic model of Leung *et al.* (1990), hereafter referred to as the LPMA model. To fit the data of Tritscher *et al.* (1992) and Sewall *et al.* (1993), the LPMA model has been modified as described below. A flowchart of the resulting model is given in Fig. 1.

TCDD in the gut compartment is periodically increased by orally administered TCDD according to the experimental schedule (Tritscher *et al.*, 1992). TCDD is extracted from the gut into the blood compartment, where some of it binds to unspecified serum protein. From the blood it is distributed to the tissue compartments.

TCDD in the liver is distributed between "metabolically available" and protein-bound pools. The latter category includes binding to CYP1A2 and to the Ah receptor, which is presumed to mediate all of the tissue's responses to TCDD. Unbound TCDD is converted to metabolites which are either transferred to the gut via the bile or released into the blood. The metabolites are treated as partitioning between the liver and blood in the same manner as TCDD. Gut metabolites ultimately appear in the feces, and blood metabolites appear in the urine. TCDD is also cleared from the liver by lysis of dead cells consequent to prolonged exposure.

Equations for the pharmacodynamic effects of TCDD described above were also included in the NIEHS model. Proteins whose synthesis is represented in the model are removed by proteolysis or, in the case of the EGF receptor, by endocytosis.

In many cases quantities that were reported in a variety of units had to be converted to those units (nanomoles of material, volume in liters, time in days) used in the model. These calculations were performed using conversion factors of 100 mg cytosolic protein/g liver (Poland and Knutson, 1982), 18 mg microsomal protein/g liver (based on average yield of membrane protein; Tritscher *et al.*, 1992), and 4.2 mg plasma membrane protein/g liver (based on average yield of membrane protein; Sewall *et al.*, 1993). Rates obtained from *in vitro* measurements conducted at temperatures other than body temperature were adjusted to 37°C using a doubling of the rate for every 10°C increase in temperature, a factor which is typical of enzymatic reactions.

The animals used in these experiments (Tritscher *et al.*, 1992) grew, on average, from 220 to 390 g during the 31 weeks of the investigation. To account for the dilution of material with growth the parameters in the model were expressed per liter in the appropriate compartment. Because of the increase in compartment volume, a steady-state concentration of a protein whose synthesis is represented in the model actually corresponds to an increase in the tissue content of the protein with time.

TCDD distribution and clearance. The LPMA model is said to be flow-limited; the rate of uptake by tissues is limited by the rate of delivery via the blood. In such a model, material is transferred from the arterial blood to various tissues at a rate given by

$$\frac{\text{Blood Content} \times \text{Flow}}{\text{Blood Volume}},$$

where Flow represents the rate of blood flow through the particular tissue. Transfer of material from the tissue to the venous blood is given by

$$\frac{\text{Tissue Content} \times \text{Flow}}{\text{Tissue Volume} \times \text{Partition Coeff}},$$

where the partition coefficient is the ratio of tissue to blood concentrations at equilibrium. The LPMA model includes compartments for gut, blood, fat, muscle (plus skin), liver, and other viscera. The cell membranes of these tissues constitute a barrier to transport of TCDD. In the NIEHS model the rates of transport given by the above expressions were multiplied by a "diffusion coefficient" to account for the membrane permeability. Different compartments were assigned different coefficient values as required to match the time course data of Abraham *et al.* (1988). For these calculations the dose was absorbed directly into the blood from the site of injection with a scaled rate constant of 0.37 $kg^{0.75}$/day, bypassing the gut compartment.

The observed blood levels of TCDD (A. M. Tritscher, unpublished results) were an order of magnitude smaller than those predicted by the LPMA model. The LPMA model includes binding of TCDD to unspecified blood lipoproteins and uses a factor of 2.5 for the ratio of protein-bound to free TCDD in the blood. This constant was replaced in the

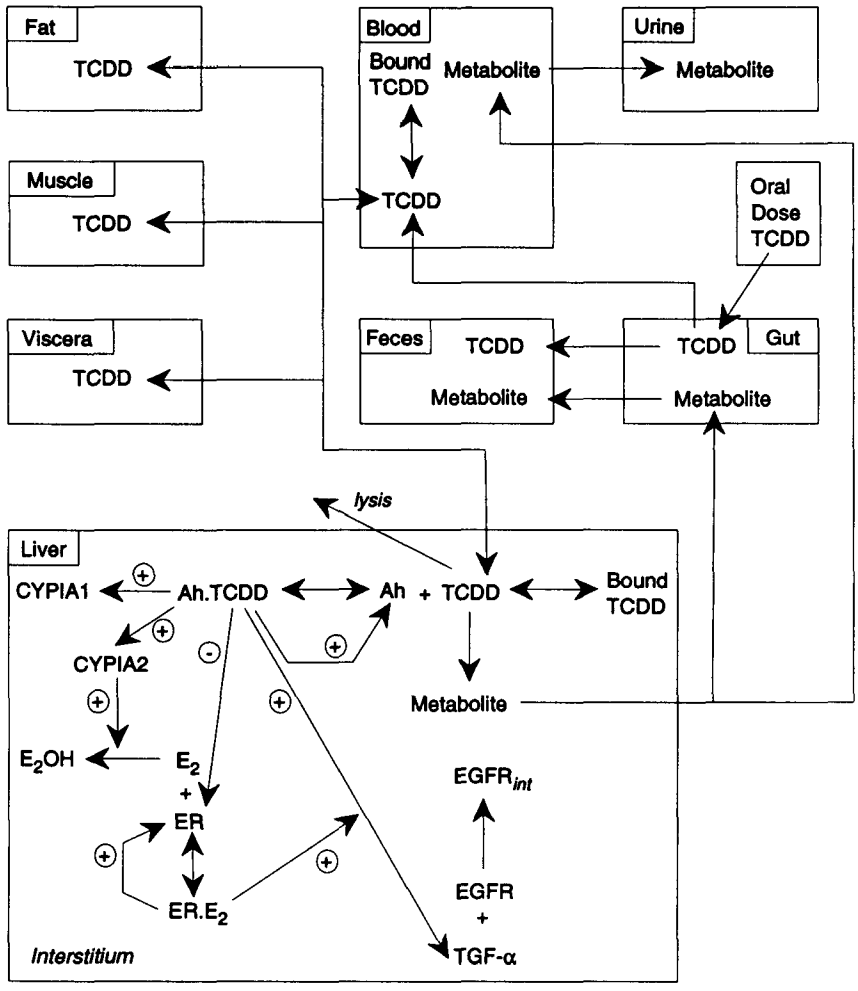

FIG. 1. Flowchart of the NIEHS model of TCDD distribution and consequent effects on gene expression in the rat liver.

NIEHS model by a reverse hyperbolic function (Appendix 1) for the maximal amount of TCDD-binding blood protein. Although the form of this equation suggests that TCDD decreases the hepatic production of blood lipoprotein, the equation is intended as just an empirical relationship.

TCDD has been found to bind to CYP1A2 in rat liver (Voorman and Aust, 1989) and P450d in the mouse liver (Poland *et al.*, 1989) with an apparent K_d of 30 nM. The estradiol 2-hydroxylase activity of this cytochrome is inhibited as a consequence of this binding (Voorman and Aust, 1987). As Poland *et al.* (1989) concluded that TCDD congeners bind at or near the active site on this enzyme, such binding is treated as competitive against estradiol in the NIEHS model. It was further assumed that TCDD binds only to the CYP1A2 that is not complexed with NADPH:cytochrome P450 reductase.

The appearance of unabsorbed TCDD in feces and TCDD metabolite(s) in both feces and urine is included in the NIEHS model. Birnbaum *et al.* (1980) administered a single oral dose of ^{14}C-labeled 2,3,7,8-tetrachlorodibenzofuran (TCDF) to rats. They calculated rate constants of 0.381 day^{-1} for the appearance of metabolites in the feces and 0.536 day^{-1} for the appearance of metabolites in the urine. However, the calculated rate constant for clearance of radiolabel from the blood during the first 3 hr in their experiments (32 day^{-1}) was an order of magnitude higher than after 3 hr (0.6 day^{-1}). Because excretion of metabolites of TCDF is limited by their rates of formation, the rate constants for clearance at late time most likely reflect this limitation. This notion is supported by nearly identical calculated rate constants for clearance of radiolabel from liver and from blood after 3 hr (Birnbaum *et al*, 1980). The higher rate constant for clearance at early time more likely reflects the rate of uptake of labeled material from the blood by the various tissues, which therefore cannot be rate limiting for clearance. The rates of transport of TCDF's and TCDD's metabolites across cell membranes should be similar. This leads to the conclusion that the specific rates of transport of TCDD's metabolites from liver to bile and from blood to urine must be larger than the rate constants for net clearance for times greater than 3 hr. Therefore, the above rate constants were increased by a factor of 10 for this model to ensure that clearance of metabolites is not limited by transport.

Rose *et al.* (1976) found as much TCDD in the livers of rats after 7 weeks of chronic dosing as Tritscher *et al.* (1992) found after 31 weeks at comparable doses. A preliminary version of the NIEHS model indicated that parameter values which enabled the model to reproduce the results of Tritscher *et al* (1992) would uniformly underestimate the results of Rose *et al.* (1976) by about 50%. The preliminary model included a first-order rate constant for metabolism of TCDD whose value could not be altered to fit the data of Rose *et al* (1976) without destroying the fit to the data of Tritscher *et al* (1992). Therefore, an additional mechanism for clearance from the liver was necessary to permit fitting both data sets simultaneously.

The cellular proliferation rate in the livers of rats exposed to biweekly doses of 1.4 μg/kg of TCDD for 30 weeks is 7.3% of the liver cells/week (Lucier *et al.*, 1991). At this rate, one would expect a 226% increase in the

weight of the liver over the course of the experiment. Only a 90% increase was observed (Lucier *et al.*, 1991; A. M. Tritscher, unpublished results), suggesting that toxic effects of cumulative exposure to TCDD result in cell death. The NIEHS model includes loss of TCDD from the liver by lysis of dead cells. The specific rate of clearance by cell lysis was assumed to increase as a hyperbolic function of the cumulative exposure to unbound liver TCDD (Appendix 1). No information regarding the fate of TCDD from lysed cells is available. Therefore, this feature of the model merely represents the net contribution to clearance by cell turnover.

TCDD-induced changes in gene expression. The complex formed by binding of TCDD to the Ah receptor has been clearly implicated in induction of CYP1A1 (Fisher *et al.*, 1990). The liganded Ah receptor modulates expression of CYP1A1 by forming a ternary complex with at least one other transcription factor (Hoffman *et al.*, 1991), and this complex then binds to enhancer sequences for the CYP1A1 gene. This mechanism was used as a prototype for all Ah receptor-dependent protein synthesis in the model. There are no data for the concentrations of the additional transcription factors required for expression nor for the competition among various receptors for binding to them. Therefore, the NIEHS model combines binding of additional transcription factors to the Ah–TCDD complex into the equations for the rate of Ah-dependent induction by treating induction of this protein as proceeding with saturation kinetics subsequent to binding of the Ah–TCDD complex itself to the appropriate enhancer sequences. That is, the Ah–TCDD complex was treated as though it were the "substrate" of the rate-limiting reaction in expression.

TCDD increases the liver concentration of the Ah receptor itself (Poland and Knutson, 1982; Sloop and Lucier, 1987). In the NIEHS model, increased synthesis of the Ah receptor is postulated to occur by an Ah-dependent mechanism similar to that outlined above. Bradfield *et al.* (1988) observed the Ah receptor's K_d for 2-iodo-7,8-dibromodibenzo-*p*-dioxin to decline from 0.16 to 0.012 nM on 32-fold dilution of the Ah receptor protein. A dissociation equilibrium constant depends on ligand solubility, pH, ionic strength, etc. The hydrophobicity of TCDD apparently makes the apparent K_d very sensitive to the above conditions. Therefore, the model uses the value of 0.27 nM reported by Poland and Knutson (1982) for the K_d of TCDD under conditions more representative of the intracellular milieu.

A rate law as described above treats the synthesis of gene product as occurring by a single event. The appearance of gene product is actually the result of many steps and requires some time before measurable amounts of protein are synthesized. The data of Sloop and Lucier (1987) show that 6 hr elapses between administration of TCDD and increased production of Ah receptor protein. To account for such a delay, the concentration of the Ah–TCDD complex at time $t - 6$ hr is substituted into the rate law to compute the rate of synthesis of protein at time t. The same approach was used for binding of all receptor complexes in all gene expression processes in the model. The model's predictions were observed to be fairly insensitive to reasonable numerical values of this delay.

Fisher *et al* (1990) found four Ah-responsive enhancer sites associated with the CYP1A1 structural gene. This finding raises the possibility of cooperative binding of Ah–TCDD complexes to these binding sites and, by analogy, to the other Ah–TCDD binding sites postulated in the model. To test for such cooperativity, Hill exponents were included in the rate equations for protein synthesis and those values which would enable the model to fit experimental observations were identified.

Proteins in the model were treated as being removed from the system with first-order kinetics. The rate constant for the proteolytic degradation of all proteins whose synthesis is represented in this model was determined from the data of Lucier *et al.* (1972).

Effects of TCDD on epidermal growth factor receptor. Choi *et al.* (1991) found TCDD to induce expression of TGF-α by an Ah-dependent mechanism in keratinocyte cultures. The TGF-α produced was exported to the extracellular fluid. A similar mechanism has been included in the NIEHS model, representing the product peptide as appearing in the interstitial space of the liver.

Intact female rats subjected to 30 weeks of oral dosing with 125 ng TCDD/kg body weight/day show a 65% reduction in the maximal binding capacity (B_{max}) of the EGF receptor in liver plasma membranes (Sewall *et al*, 1993) at a liver TCDD content of 0.063 nmol/g (Tritscher *et al.*, 1992). Ovariectomized rats exhibit only a 19% reduction of EGF B_{max} at a liver TCDD content of 0.106 nmol/g after 30 weeks of oral dosing at 100 ng TCDD/kg/day (Clark *et al.*, 1991). Sunahara *et al.* (1989) observed a 56% reduction in the B_{max} in female rats 10 days following gavage with 10 μg TCDD/kg. Madhukar *et al.* (1984) observed a 45% decline in the B_{max} of the EGF receptor of male rats 10 days after a single intraperitoneal injection of 10 μg TCDD/kg. The NIEHS model predicts a liver TCDD content of 0.217 nmol/g for females and 0.223 nmol/g for males at the end of the 10-day period. The responses of males, ovariectomized females, and intact females may indicate different sensitivities for transcriptional activation of the TGF-α gene by the Ah–TCDD complex as a consequence of differences in the amount of hepatic estrogen (or some other ovarian hormone). Intact females, having much more circulating estrogen than either males or ovariectomized females, should be the most sensitive.

Both the Ah–TCDD and estrogen receptor–estrogen (ER–E) complexes may be acting as transcriptional activators of the TGF-α gene, but a synergistic interaction between them appears to be necessary to fully activate transcription. In the NIEHS model, binding of the ER–E complex is treated as activating the TGF-α gene for subsequent binding of the Ah–TCDD complex. However, binding of this complex to the TGF-α gene has not been proven, and other mechanisms are possible.

Estrogen metabolism and the production of the estrogen receptor are included in the NIEHS model to account for the effects of estrogen on alterations in the EGF receptor. The kinetic constants for estradiol 2-hydroxylase activity (Appendix 2) would predict an increase in enzymatic activity comparable to the ninefold rise in CYP1A2 resulting from exposure to TCDD. Only a threefold increase in activity was actually observed (Graham *et al.*, 1988) in female rats. The smaller increase in activity was attributed to rate limitation by the availability of NADPH:cytochrome P450 reductase.

This reductase forms a complex with cytochrome P450 (Coon *et al*, 1977). The complex binds molecular oxygen and is reduced by NADPH to eliminate water, leaving a single oxygen atom to combine with substrate (Estabrook and Werringloer, 1977). As all P450 isozymes compete for binding to reductase, there might not be sufficient reductase to bind to all the increased CYP1A2. Indeed, there is evidence (Ullrich and Duppel, 1975) that P450 reductase is limiting for other mono-oxygenase reactions that are not induced. Therefore, binding of the reductase to both CYP1A1 and CYP1A2 was included in the NIEHS model. Estradiol and reductase were allowed to bind to CYP1A2 in random order, and oxygen and NADPH were treated as saturating.

The catechol estrogens produced by the activity of CYP1A2 bind to the estrogen receptor an order of magnitude more weakly than does estradiol (Li *et al*, 1985). Such binding is included in the NIEHS model, and the resulting complex was allowed to function identically to the ER–estradiol complex. This means that the ER–estradiol and ER–catechol estrogen complexes produce the same effect on gene expression, but that 10 times as much catechol estrogen as estradiol is required to achieve the same quantitative response. Therefore, the symbol ER–E is used in this report to represent complexes between the estrogen receptor and either estradiol or catechol estrogen.

The estrogen-binding capacity of the estrogen receptor in the livers of ovariectomized or immature female rats is increased by administration of estradiol (Romkes *et al.*, 1987). One interpretation of this observation is that there is synthesis of additional receptor protein consequent to binding of the ER–E complex to the estrogen receptor gene. Female rats treated with 100 ng TCDD/kg/day exhibit a 55% reduction in the estrogen receptor (Clark *et al*, 1991). A 40% reduction in estrogen receptor levels was

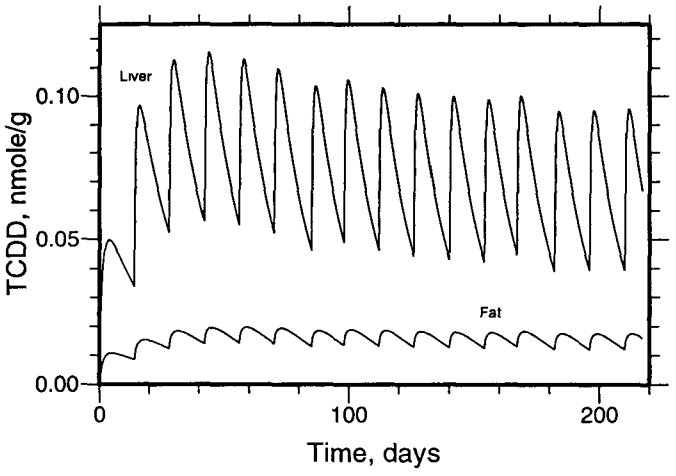

FIG. 2. Computed time courses of the accumulation of TCDD in liver and fat of rats treated with 125 ng TCDD/kg body weight/day.

observed in the livers of mice exposed to a single 100 ng/kg dose of TCDD (Lin *et al.*, 1991b). "Ah-unresponsive" mice having defective or insufficient Ah receptors require higher TCDD exposures than do normal mice to achieve a given reduction in estradiol binding capacity (Lin *et al.*, 1991b). These observations may be interpreted as indicating that induction of estrogen receptor is inhibited by binding of the Ah–TCDD complex at another DNA binding site. This mechanism is represented in the NIEHS model as noncompetitive inhibition of receptor synthesis by the Ah–TCDD complex (Appendix 1).

The NIEHS model was implemented in the SCoP simulation program (Kootsey *et al.*, 1986; Kohn *et al.*, 1993). Values for parameters that could not be obtained from the literature were adjusted to make the model reproduce the observations of Tritscher *et al.* (1992) and Sewall *et al.* (1993). Where experimental data were available, parameters were estimated using the "praxis" algorithm (Brent, 1973) in the SCoPfit program (part of the SCoP package).

The fully assembled model was composed of 33 first-order ordinary differential equations. There were 77 constants in these equations, of which 15 were freely adjustable parameters. The remaining 62 constants were obtained from the literature, constrained by experimental data, or mathematically determined by the law of conservation of mass. The model's equations are listed in Appendix 1, and all parameter values (estimated or from the literature) are given in Appendix 2. The experiments of Tritscher *et al.* (1992) and Sewall *et al.* (1993) were simulated by integration of the differential equations to 217 days of biweekly exposure to TCDD using a C language translation of the LSODA Gear integration package from Lawrence Livermore Laboratory.

RESULTS

The NIEHS model predicts that 92.5% of the ingested TCDD is absorbed into the bloodstream and 7.5% appears unchanged in the feces. As some of the TCDD cleared from the liver by cell lysis may be returned to the gut (the model does not treat the fate of this material), this result may be an underestimation of the amount of TCDD in the feces. Rose *et al.* (1976) report an average value of 90.3% of ^{14}C-TCDD extracted from the guts of female rats. The model predicts that 92.2% of the metabolite appears in the feces and 7.8% appears in the urine at a dose of 125 ng/kg/day. The dose of TCDD did not have a significant effect on these predictions. Urinary radioactivity at doses comparable to those of Tritscher *et al.* (1992) was often undetectable in the chronic dosing experiments of Rose *et al.* (1976).

The computed time courses for TCDD in the liver and fat for biweekly oral doses of 125 ng/kg body weight/day (Tritscher *et al.*, 1992) and for a single subcutaneous injection of 300 ng/kg (Abraham *et al.*, 1988) are given in Figs. 2 and 3, respectively. From the fit to the data of Abraham *et al.* (1988) the NIEHS model predicts an initial half time of 11.8 days for clearance of TCDD from the liver and an overall liver half time of 13.5 days. Abraham *et al.* (1988) report an initial half time of 11.5 days and an overall half time of 13.6 days in the liver. The model predicts a half time of 22.3 days in the fat; Abraham *et al.* (1988) report a half time of 24.5 days in fat.

The predicted dose dependency of TCDD concentration in the blood, liver, and fat after 217 days of exposure (biweekly oral doses of TCDD for 31 weeks) is given in Table 1. The NIEHS model predicts a linear relationship between administered dose and the concentration in the liver at doses between 3.5 and 125 ng/kg/day (Fig. 4) in agreement with the experimental observations (Tritscher *et al.*, 1992). Table 2 gives the model's predictions for repeated doses, and Table 3 gives the model's predictions for single doses.

The Hill exponents in the equations for the rates of induction of CYP1A1 and CYP1A2 were estimated from the

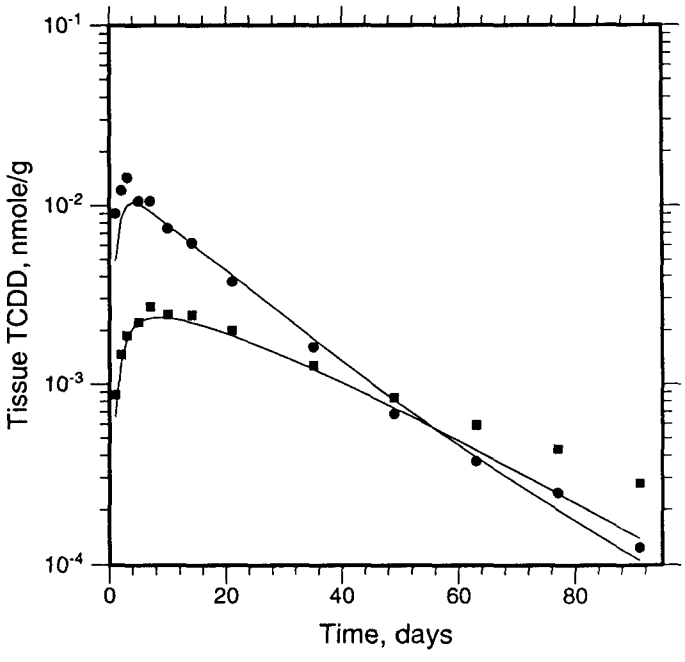

FIG. 3. Fit to the time courses in rats given a subcutaneous injection of 300 ng/kg body weight of TCDD (Abraham *et al.*, 1988). Circles are for observations in liver, and squares are for observations in fat.

TABLE 1
Computed TCDD Distribution

Dose, ng/kg/day	Blood, nM[a]	Liver, nmol/g[b]	Fat, nmol/g
0.01	0.0000967	0.00000394	0.00000432
0.1	0.000925	0.0000395	0.0000421
0.3	0.00254	0.000118	0.000120
1.0	0.00672	0.000389	0.000350
3.5	0.0150	0.00142	0.000954
	(0.0127–0.0174)	(0.000652–0.00267)	
7.0	0.0218	0.00313	0.00162
10.7	0.0269	0.00515	0.00223
	(0.0143–0.0251)	(0.00435–0.00683)	
15.0	0.0315	0.00754	0.00289
20.0	0.0361	0.0105	0.00361
25.0	0.0400	0.0134	0.00431
30.0	0.0435	0.0165	0.00496
35.7	0.0471	0.0199	0.00568
	(0.0323–0.103)	(0.0155–0.0311)	
45.0	0.0523	0.0252	0.00683
55.0	0.0573	0.0307	0.00804
65.0	0.0618	0.0360	0.00925
75.0	0.0661	0.0417	0.0104
85.0	0.0700	0.0468	0.0115
100.0	0.0754	0.0543	0.0133
115.0	0.0806	0.0618	0.0149
125.0	0.0839	0.0673	0.0160
	(0.0401–0.134)	(0.0385–0.134)	

[a] Range of observed values (A. M. Tritscher, unpublished results) is given in parentheses.

[b] Range of observed values (Tritscher *et al*, 1992) is given in parentheses.

TABLE 2
Fit of Model to Observed TCDD Concentrations Following Repeated Doses[a]

	Dose, ng/kg		
	10	100	1000
	Dosing 5 days/week for 1 week		
Liver	0.000898	0.0141	0.147
	(0.0)	(0.00870–0.0155)	(0.143–0.165)
Fat	0.0004063	0.00302	0.0249
	(0.0)	(0.000932–0.00466)	(0.0217–0.0404)
	Dosing 5 days/week for 3 weeks		
Liver	0.00235	0.0373	0.302
	(0.00217–0.00280)	(0.0298–0.0435)	(0.227–0.457)
Fat	0.00108	0.00726	0.0586
	(0.000932)	(0.0059–0.0109)	(0.0497–0.0963)
	Dosing 5 days/week for 7 weeks		
Liver	0.00408	0.0505	0.329
	(0.00342–0.00652)	(0.0519–0.0711)	(0.471–0.796)
Fat	0.00170	0.0107	0.0766
	(0.000620–0.00124)	(0.0118–0.0161)	(0.0767–0.305)

[a] Concentrations in nmol/g tissue; observed values (Rose *et al.*, 1976) are in parentheses.

data of Tritscher *et al.* (1992). Hill exponents of 1.0 for induction of CYP1A1 (Fig. 5) and CYP1A2 (Fig. 6) reproduced the observed responses. Because binding of TCDD to the Ah receptor is the initial step in the induction of cytochrome P450 isozymes, the fractional occupancy of the receptor will have a strong effect on the predictions of the model. As fractional occupancy is dramatically affected by cooperativity in ligand binding, estimation of the Hill exponent for this binding was included in the course of estimating the gene induction parameters for CYP1A1 and CYP1A2. The optimal value of the exponent was 0.96, indicating a lack of cooperativity (i.e., hyperbolic binding). This value agrees with the estimate of 1.0 obtained by Gasiewicz (1984) and by Bradfield *et al.* (1988).

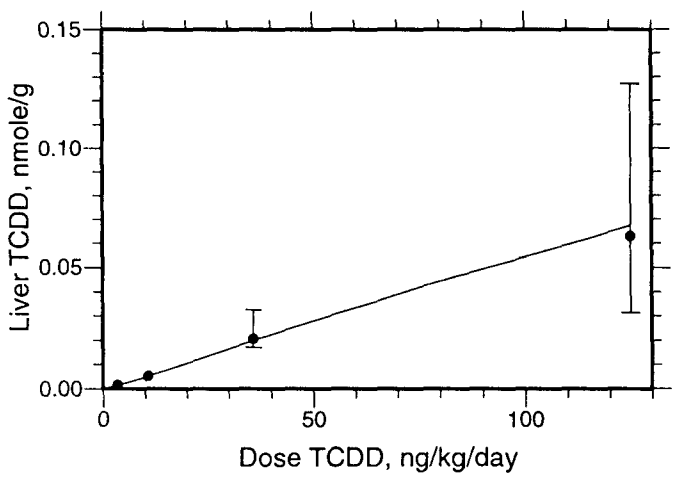

FIG. 4. Computed relationship between liver TCDD concentration and administered dose. Experimental data points (filled circles) are the average values of Tritscher *et al.* (1992). Error bars denote the range of observed values.

Table 4 compares the NIEHS model's predictions to the observed terminal concentrations of the Ah receptor and of the induced P450 isozymes. The model predicts that the fractional occupancy of the Ah receptor by TCDD (not included in Table 4) rises from 13.4% at a dose of 3.5 ng TCDD/kg/day to 69.3% at 125 ng/kg/day. The liver concentration of CYP1A1 is 0.02 nmole/g in the absence of administered TCDD (Tritscher *et al.*, 1992), consistent with (but not proof of) the notion of an endogenous ligand or a ligand of dietary origin for the Ah receptor. A liver concentration of 1.5 pM (in TCDD equivalent units) of this ligand reproduces the observed basal level of CYP1A1. Needham *et al.* (1991) detected a background level of TCDD of 2 pM in human livers.

Table 5 gives the predicted concentration of interstitial TGF-α after 217 days of exposure to TCDD and the conse-

TABLE 3
Fit of Model to Observed TCDD Concentrations Following Single Doses[a]

Dose, ng/kg	Liver, nmol/g	Fat, nmol/g
	22 days following gavage[b]	
1000	0.0114	0.00389
	(0.00869–0.0145)	(0.0010–0.0222)
	4 days following gavage[c]	
0.25	0.00000366	
	(0.0000035)	
0.5	0.00000734	
	(0.0000035)	
1	0.0000147	
	(0.0000078)	
10	0.000155	
	(0.000066)	
100	0.00198	
	(0.0011)	
	7 days following subcutaneous injection[d]	
1	0.0000119	0.0000101
	(0.0000096)	(not detected)
3	0.0000365	0.0000302
	(0.000032)	(0.000043)
10	0.000129	0.0000995
	(0.000126)	(0.000153)
30	0.000456	0.000290
	(0.000503)	(0.000432)
100	0.00218	0.000885
	(0.00217)	(0.00104)
300	0.00928	0.00234
	(0.0105)	(0.0025)
1000	0.0377	0.00699
	(0.0332)	(0.0063)
3000	0.116	0.0201
	(0.087)	(0.0114)

[a] Observed values are in parentheses.
[b] Rose *et al.* (1976).
[c] J. Vanden Heuvel, NIEHS, personal communication.
[d] Abraham *et al.* (1988).

quent distribution of the EGF receptor between the plasma membrane and cytosol. Figure 7 gives the decline in EGF binding capacity of the plasma membranes with the dose of TCDD and corresponds to that fraction of the receptor which had not been internalized consequent to binding of TGF-α. Figure 8 relates the predicted degree of internalization of the EGF receptor to the TGF-α concentration. The points on this curve corresponding to the predicted responses for the four dose groups of Sewall *et al.* (1993) are also shown. Note that the TGF-α concentration is given in pmol/g liver; multiplying by 10 gives the concentration in the interstitial fluid in nanomolar. These interstitial concentrations are comparable to the concentrations produced by TCDD in the extracellular fluid of keratinocyte cultures exposed to TCDD (Choi *et al.*, 1991). These results were

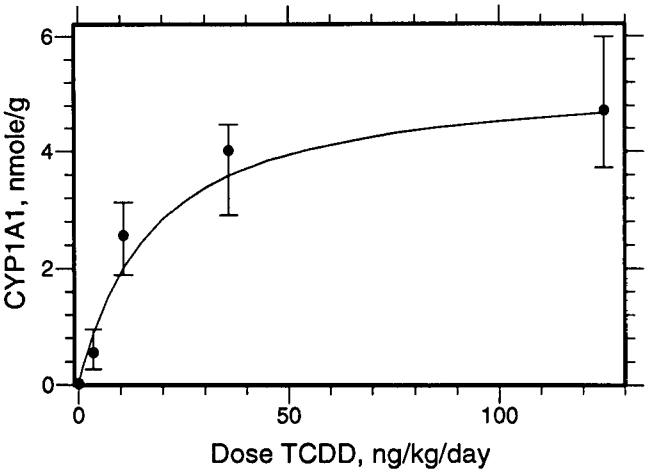

FIG. 5. Induced CYP1A1 concentration vs administered dose of TCDD. Experimental data points (filled circles) are the average values of Tritscher *et al.* (1992). Error bars denote the range of observed values.

obtained with a model which required prior binding of the ER–E complex to activate binding of the Ah–TCDD complex to the TGF-α gene. An alternative model which postulates random-order binding of the ER–E and Ah–TCDD complexes produced quantitatively similar results.

The NIEHS model predicts that 10 days after administration of a single dose of 1 μg TCDD/kg there should be a 20.1% decrease in the B_{max} of the plasma membrane EGF receptor in female rat livers (14% observed; Sunahara *et al.*, 1989), but only a 9.6% decrease in male rat livers (10% observed on average; Madhukar *et al.*, 1984). This computed twofold greater responsiveness of the female over the male persisted over all doses in the range of 0.1 to 10 μg/kg.

As estrogen plays a major role in regulating TCDD's effects on the EGF receptor, estrogen action was examined in

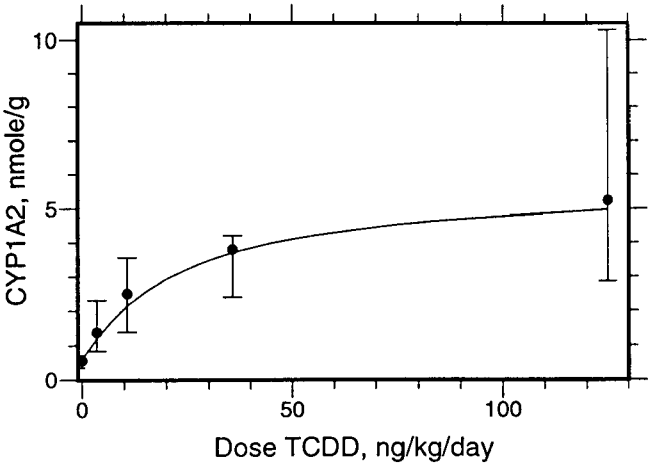

FIG. 6. Induced CYP1A2 concentration vs administered dose of TCDD. Experimental data points (filled circles) are the average values of Tritscher *et al.* (1992). Error bars denote the range of observed values

TABLE 4
Ah Receptor and Induced Cytochrome P450 Isozymes

Dose, ng/kg/day	Ah receptor, pmol/g	CYP1A1, nmol/g[a]	CYP1A2, nmol/g[a]
0.0	2.09	0.0234	0.557
	(2.1)[b]	(0.0081–0.0351)	(0.352–0.714)
	(2.1–3.2)[c]		
0.01	2.10	0.0271	0.561
0.1	2.14	0.0591	0.583
0.3	2.23	0.126	0.628
1.0	2.45	0.328	0.771
3.5	3.21	0.881	1.190
		(0.269–0.953)	(0.840–2.31)
7.0	4.01	1.501	1.699
10.7	4.68	2.011	2.145
	(3.0–5.7)[c]	(1.89–3.13)	(1.39–3.56)
15.0	5.25	2.443	2.544
20.0	5.77	2.841	2.927
25.0	6.16	3.135	3.224
30.0	6.50	3.377	3.476
	(5.5–7.8)[c]		
35.7	6.80	3.589	3.704
		(2.91–4.47)	(2.39–4.21)
45.0	7.16	3.845	3.990
55.0	7.43	4.038	4.211
65.0	7.63	4.183	4.380
75.0	7.82	4.314	4.537
85.0	7.95	4.409	4.652
100.0	8.11	4.519	4.785
	(4.9–10.5)[c]		
115.0	8.24	4.612	4.899
125.0	8.32	4.672	4.975
	(8.5)[d]	(3.72–5.99)	(2.86–10.3)

[a] Experimental data from Tritscher *et al* (1992) are given in parentheses.
[b] Poland and Knutson (1982).
[c] Sloop and Lucier (1987).
[d] Maximal induction from Poland and Knutson (1982).

TABLE 5
Effects of TCDD on the EGF Receptor

Dose, ng/kg/day	EGF receptor, pmol/g[a]	TGF-α, pmol/g	Internalized EGF receptor, pmol/g
0.0	2.553	0.0	0.0
	(2.06–2.61)		
0.01	2.553	0.00000995	0.000849
0.1	2.545	0.0000999	0.00854
0.3	2.53	0.000291	0.0246
1.0	2.48	0.000901	0.0747
3.5	2.34	0.00280	0.219
	(1.45–2.95)		
7.0	2.16	0.00540	0.391
10.7	2.01	0.00806	0.541
	(1.18–2.45)		
15.0	1.88	0.0107	0.674
20.0	1.75	0.0138	0.807
25.0	1.65	0.0165	1.00
30.0	1.55	0.0194	
35.7	1.46	0.0222	1.09
	(0.899–1.64)		
45.0	1.36	0.0263	1.20
55.0	1.28	0.0299	1.28
65.0	1.22	0.0326	1.34
75.0	1.15	0.0361	1.40
85.0	1.11	0.0386	1.44
100.0	1.07	0.0429	1.49
115.0	1.02	0.0446	1.53
125.0	0.990	0.0470	1.56
	(0.702–1.13)		

[a] Experimental data from Sewall *et al.* (1993) are given in parentheses.

the model. Chronic exposure to TCDD was found to reduce the concentration of the hepatic estrogen receptor in DEN-initiated female rats from 5.1 to 2.3 pmol/g (Clark *et al.*, 1991). The model reproduces this decrease in the estrogen receptor level (Table 6). It also predicts that the reduction is dependent on the dose of TCDD with a curve shape similar to that obtained for mouse liver by Lin *et al.* (1991b).

The calculated liver concentrations of estradiol and catechol estrogen and the calculated rate of the estradiol 2-hydroxylase activity of CYP1A2 at various doses are given in Table 6. At a dose of 125 ng TCDD/kg/day the calculated rate of the estradiol 2-hydroxylase is 3.1-fold greater than in the absence of administered TCDD whereas CYP1A2 is computed to increase 8.9-fold over its basal level. The NIEHS model predicts that only 44.9% of the CYP1A2 is complexed with reductase in the absence of administered TCDD. This percentage decreases slightly as more enzyme is induced; at a dose of 125 ng/kg/day, 39.8% of CYP1A2 is calculated to be complexed with reductase. The lack of stoichiometric amounts of bound reductase is consistent with

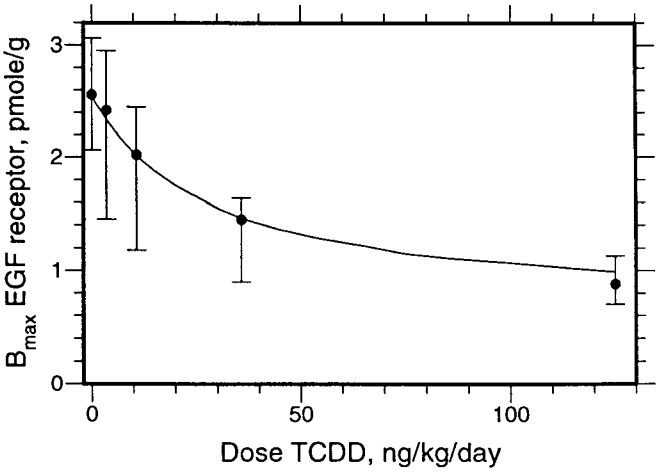

FIG. 7. Reduction of B_{max} of EGF receptor vs administered dose of TCDD. Experimental data points (filled circles) are the average values of Sewall *et al* (1993). Error bars denote the range of observed values.

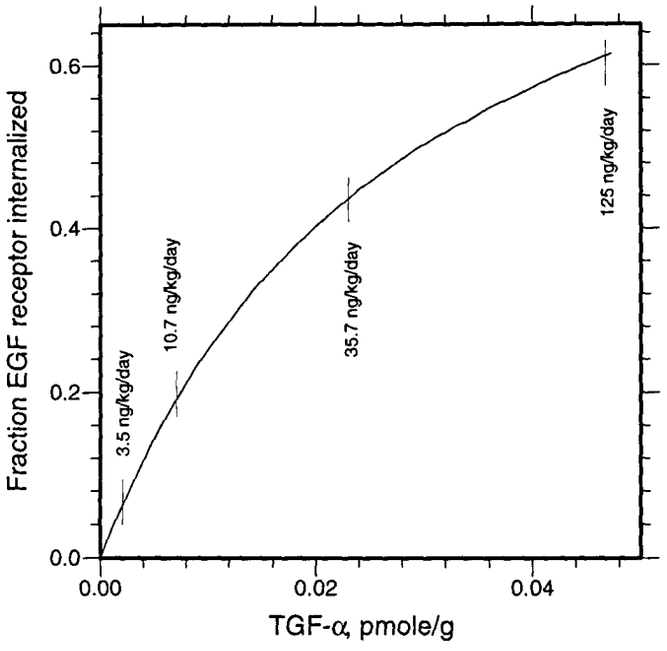

FIG. 8. Calculated fraction of EGF receptor redistributed from the plasma membrane into the cytosol as a function of the concentration of TGF-α. Vertical bars indicate model's predictions for the four dose groups of Sewall *et al.* (1993).

the conclusion of Graham *et al.* (1988) that P450 reductase is limiting for the hydroxylation of estradiol. However, at a dose of 125 ng/kg/day, the estradiol concentration in the liver is computed to fall to 39% of its level in the absence of administered TCDD. As the computed estradiol concentration is below its K_m for this enzyme, the enzymatic rate is probably limited more by depletion of substrate than by availability of reductase.

DISCUSSION

The purpose of this modeling effort was to suggest plausible biochemical mechanisms which may explain the observed tissue effects of TCDD in female rats. Internalization of the EGF receptor in response to induction of TGF-α is a possible origin for the mitogenic signal important to carcinogenesis. The NIEHS model's prediction of TGF-α concentrations that are comparable to those observed in cell cultures is consistent with this mechanism, and the model's success in reproducing observed responses to TCDD supports the proposed mechanism. However, other EGF-like peptides may be involved although we have treated TGF-α as the nominal ligand for our modeling. In addition, the production of a considerable quantity of catechol estrogens may play a role in carcinogenesis (Li *et al.*, 1985) either by covalent binding to protein or DNA (Tsibris and McGuire, 1977) or by generation of active oxygen species (Cerutti, 1978). The proliferative effect of EGF receptor-mediated events could also provide genetically altered cells with a selective growth advantage. This mechanism is consistent with the general agreement that TCDD is a tumor promoter, but not an initiator, in two-stage models for hepatocarcinogenesis (Kociba *et al.*, 1978; Pitot and Sirica, 1980; Lucier *et al.*, 1991) and a complete carcinogen in the 2-year chronic animal bioassay (Huff *et al.*, 1991).

EGF Receptor and Cellular Proliferation

Figure 7 shows the predicted and observed responses of the maximal binding of EGF to liver plasma membranes vs dose of TCDD. Although the liver TCDD concentration is linear in the administered dose over the range 3.5–125 ng/kg/day, the response of the EGF receptor resembles a hyperbolic curve. As this response may be involved in the mechanism of tumorigenesis in TCDD-treated rats, it would be expected that it would correlate with tumor incidence better than does tissue dose. If this is true, linear extrapolation of effects at high doses to low doses by a line that passes through the background incidence would underestimate low-dose effects. However, such hyperbolic curves are approximately linear in the low-dose range, indi-

TABLE 6
Effects of TCDD on Estrogen Metabolism in Liver

Dose, ng/kg/day	Estrogen receptor, pmol/g[a]	Estradiol, pmol/g	Catechol estrogen, pmol/g	Estradiol 2-hydroxylase, nmol/g/day
0.0	5.19 (5.1)	0.154	0.610	0.0347
0.01	5.19	0.154	0.613	0.0348
0.1	5.17	0.153	0.633	0.0359
0.3	5.12	0.151	0.672	0.0381
1.0	4.98	0.145	0.785	0.0446
3.5	4.59	0.130	1.067	0.0605
7.0	4.18	0.114	1.336	0.0757
10.7	3.85	0.103	1.517	0.0860
15.0	3.58	0.0962	1.641	0.0931
20.0	3.33	0.0878	1.734	0.0984
25.0	3.16	0.0828	1.792	0.102
30.0	3.01	0.0792	1.828	0.104
35.7	2.88	0.0760	1.854	0.105
45.0	2.73	0.0722	1.877	0.106
55.0	2.62	0.0692	1.888	0.107
65.0	2.54	0.0670	1.893	0.107
75.0	2.46	0.0653	1.896	0.107
85.0	2.41	0.0638	1.895	0.107
100.0	2.35	0.0642	1.891	0.107
115.0	2.30	0.0610	1.889	0.107
125.0	2.26 (2.3)	0.0601	1.888	0.107

[a] Experimental data of Clark *et al* (1991) are given in parentheses.

cating that linear extrapolation from low doses to extremely low doses should still be valid.

Because the interstitial space occupies about 10% of the liver volume, the accumulated TGF-α is 10 times more concentrated in the interstitium than it would be in the cytosol. This concentration effect has significant consequences for the predicted reduction in the B_{max} of the EGF receptor. If the TGF-α were actually produced in some other tissue, it would have to be transported to the liver interstitium via the blood. This would greatly dilute the concentration of the peptide and require synthesis of 15 times as much material to result in the observed decrease in EGF-binding capacity of the plasma membrane.

Although production of TGF-α by the liver was modeled as being induced by the Ah–TCDD complex, there is evidence (Gaido *et al.*, 1992) for post-transcriptional regulation as well. TCDD may act to "stabilize" the mRNA for that peptide. If information regarding the mechanism of such an effect were available, it could readily be incorporated into this model. As it is, the model merely states that TCDD stimulates the rate-limiting step in the net synthesis of hepatic TGF-α.

Under normal conditions, one of the functions of TGF-α is as a signal for regenerative hyperplasia of injured tissue (Burgess, 1989; Mead and Fausto, 1989). The substrates for the internalized EGF receptor's tyrosine kinase may be involved in modulating this process, and their enzymatic activities may be regulated by phosphorylation. According to the NIEHS model, TCDD causes inappropriate stimulation of this process. Cells which had previously sustained nonlethal DNA damage could be stimulated to reproduce before the damage could be repaired. With the damage fixed in the genome, clonal expansion of the transformed cells could provide a necessary step in tumor development.

Different populations of cells may differ in the sensitivity of their proliferative responses to TCDD. The small population of polyploid cells in the livers of rats treated with DEN and ethinylestradiol are especially rich in EGF receptors (Vickers and Lucier, 1991). It is possible that these cells would show a correspondingly larger concentration of internalized EGF receptors in response to induction of TGF-α and, hence, a stronger mitogenic signal. If the population of polyploid cells includes preneoplastic cells, proliferation of these previously transformed cells may represent the most important effect of TCDD in hepatocarcinogenesis.

The NIEHS model predicts that after 31 weeks of treatment, as much EGF receptor is internalized in the male at a dose of 125 ng TCDD/kg/day as is internalized in the female at a dose of 15 ng/kg/day. This behavior is consistent with the observed lower sensitivity of the male (Madhukar *et al.*, 1984). It arises because serum estradiol in the male is about 10% of that in the female (D. Schomberg, Duke University, personal communication), resulting in concentrations of the ER–E complex which only slightly stimulates production of TGF-α. Because the male rat is predicted to produce far less TGF-α than the female, the rate of cellular proliferation in the male at low doses of TCDD should be indistinguishable from that at zero dose. This prediction of the model could explain the absence of excess liver tumors in male rats exposed to TCDD (Kociba *et al.*, 1978; National Toxicology Program, 1982).

Cytochrome P450 and Estrogen Metabolism

As estrogen is necessary for TCDD-mediated internalization of the EGF receptor, induction of CYP1A2 may also be an important index of cancer risk because this cytochrome catalyzes oxidation of estradiol to catechol estrogen. The predicted response curve for this indicator (Fig. 6) is hyperbolic. This behavior is attributable to the Hill coefficient of 1 determined for induction of this protein.

The finding of at least four enhancer sequences for induction of CYP1A1 (Fisher *et al.*, 1990) suggests the possibility that several enhancer sites must be occupied to enable transcriptional activation of the CYP1A1 gene. Such a mechanism could lead to sigmoidal response curves for the induction of this cytochrome. The Hill exponent of 1, which gave the best fit to the data of Tritscher *et al.* (1992), yields a hyperbolic response curve (Fig. 5). Because many factors other than cooperative interactions among binding sites (e.g., heterogeneous uptake of TCDD in the liver and recycling of TCDD among Ah receptor molecules) could influence the shape of the response curve, the value of the Hill exponent is not necessarily related to the number of binding sites for the Ah–TCDD complex.

Using steady-state kinetics and a simpler model, Portier *et al.* (1993) estimated Hill exponents of 1.86 for CYP1A1 induction and 0.534 for CYP1A2 induction. A possible origin of the difference in Hill exponents is the steady state model's neglect of the increase in the concentration of the Ah receptor with increasing dose of TCDD. The maximal slope of the curve for induction rate vs inducer concentration is steeper for cooperative kinetics (Hill exponents not equal to 1) than for hyperbolic kinetics. The increase in the concentration of the Ah receptor with dose can mimic the steeper rise of rate of synthesis with dose exhibited by cooperative kinetics.

Portier *et al.* (1993) showed the importance of the mechanism by which putative alternative ligands bind to the Ah receptor and/or modulate protein synthesis. In the NIEHS model, constitutive expression of all proteins except CYP1A1 was assumed to be independent of the Ah receptor. Background CYP1A1 levels were treated as being produced by an additional Ah receptor-dependent mechanism. An "independent" mechanism accentuates any sigmoidicity in the response curve. An "additive" mechanism minimizes such sigmoidicity and leads to linearity in the low-dose response. Such an effect may be involved in producing

the hyperbolic response of CYP1A1 (Fig. 5). It is also possible that CYP1A1 is constitutively expressed at a low rate by a mechanism that does not involve the Ah receptor. Additional experiments on the mechanism of expression of CYP1A1 in the absence of administered TCDD could resolve this issue.

As catechol estrogens bind more weakly to the estrogen receptor than does estradiol, it is conceivable that their production from estradiol by the estradiol 2-hydroxylase activity of CYP1A2 could result in reduced ligation of the estrogen receptor. Because it was assumed that the ER–E complex induces synthesis of the estrogen receptor protein, a reduced calculated rate of synthesis of the receptor could result from the enzymatic activity of CYP1A2. An alternative model, in which the ER–E complex was a transcriptional activator of the estrogen receptor gene, but the Ah–TCDD complex was not a transcriptional inhibitor of the gene, predicted only an 18.9% decrease in the estrogen receptor concentration. As a 55% reduction in the estrogen receptor concentration was observed (Clark *et al.*, 1991), an Ah receptor-mediated effect of TCDD on expression of the estrogen receptor such as that incorporated into the NIEHS model is likely. This result is consistent with the observation (Lin *et al.*, 1991b) of only a 10% decrease in receptor level in Ah-deficient mice.

Serum estradiol in the female rat oscillates from 15 to 120 pg/ml over a 5-day estrus cycle. Including such oscillations in the NIEHS model could make as much as a 12% difference in the model's predictions, depending on where in the cycle the simulation was begun. The serum levels, 50–60 pg/ml, reported by Shiverick and Muther (1983) are close to the average level over the entire cycle, and the experimental data are the average results for nine individuals. Therefore, use in the model of Shiverick and Muther's value for the circulating estradiol concentration is justified.

TCDD Tissue Dose

The LPMA model predicts a liver TCDD content 2.4-fold higher than observed at a dose of 125 ng/kg/day (Tritscher *et al.*, 1992). The LPMA model assumes that TCDD binds to total CYP1A2 with a K_d of 7 nM, and it predicts a liver CYP1A2 concentration 60% higher than observed at a dose of 125 ng/kg/day (Tritscher *et al.*, 1992). The resulting large calculated amount of bound TCDD is most likely responsible for the overprediction of liver TCDD.

Although the NIEHS model accurately reproduces the observed CYP1A2 concentration, much less binding had to be assumed in order to reproduce the experimental data. An alternative model in which TCDD is bound nonspecifically to an unspecified protein with K_d between 5 and 8 nM required only about 0.2 nmol/g liver of that protein to reproduce the observed liver TCDD content. Setting the binding protein concentration to values comparable to that of CYP1A2 necessitated a higher rate constant for clearance of TCDD from the liver in order to match the experimental data and resulted in a half time in the liver of only 7 days, half the experimentally observed value.

Livers of TCDD-treated rats often show an increase in lipid content. From the data of Tritscher *et al.* (1992) the relationship (fraction lipid) = 0.01865 × (nmol total liver TCDD) + 0.1443 × (kg body weight) was obtained. Allowing the TCDD to partition between the aqueous phase of the liver cytosol and lipid droplets with partition coefficients between 140 and 350 did not sequester enough TCDD to match the data without also requiring induction of TCDD-binding protein.

The dose–response relationships illustrated by the NIEHS model are important for understanding the risks of adverse health effects following exposure to TCDD. Sigmoidicity in the response requires a higher concentration to produce a given response at low dose than does hyperbolic response exhibiting the same concentration for half-maximal effect. Such behavior can have dramatic consequences for the estimated minimum exposure to TCDD that should produce an unacceptable risk of adverse health effects (Portier *et al.*, 1993). All of the amounts of protein calculated in this model show hyperbolic dependence on dose. The fact that the model presented here does not predict sigmoidicity in the observed responses indicates that the response is approximately linear at very low doses. The model's success in reproducing these responses in a biochemically realistic way suggests that it should also be useful in the analysis of more complex dose–response relationships, such as for cellular proliferation rates and tumor incidence.

APPENDIX 1: MODEL EQUATIONS

Compartment and Flow Characteristics

CardiacOutput	= 404 × BodyWeight$^{0.75}$ (Delp *et al.*, 1991)
StartWeight	= 0.237 kg (Tritscher *et al.*, 1992)
BodyWeight	= StartWeight + 0.219674 × $e^{-0.002859 \times \text{dose}}$ × time/(116.345 + time) (BodyWeight was estimated from the data of Tritscher et al. (1992) as a function of dose and time by nonlinear regression)
BloodVolume	= 0.054 × BodyWeight (Delp *et al.*, 1991)

APPENDIX 1: Continued

FatVolume = 0.08 × BodyWeight (average of literature values: Leung *et al.*, 1990; Delp *et al.*, 1991)
MuscleVolume = 0.59 × BodyWeight (Delp *et al.*, 1991) (this compartment also includes skin and other slowly perfused tissues)
VisceraVolume = 0.083 × BodyWeight (Delp *et al.*, 1991) (this compartment includes all richly perfused tissues)
LiverVolume = BodyWeight × (0.03324 + 0.01927 × dose/(88.91 + dose)) (LiverVolume was estimated from the data of Tritscher *et al.* (1992) as a function of dose by nonlinear regression)
LiverInterstitialVolume = frac_interstitium × LiverVolume
LiverFlow = 0.15 × CardiacOutput (Delp *et al*, 1991)
FatFlow = 0.07 × CardiacOutput (Delp *et al.*, 1991)
MuscleFlow = 0.36 × CardiacOutput (Delp *et al.*, 1991)
VisceraFlow = 0.40 × CardiacOutput (Delp *et al.*, 1991)

Distribution of TCDD

GutTCDD = GutTCDD + dose(t) × BodyWeight/322 (doses at time t—in ng/kg—were introduced into the gut compartment according to the experimental schedule; dose(t) = 0 if no dose is scheduled at time t)
GutTCDD′ = $-k$_absorption × GutTCDD/BodyWeight$^{0.75}$ − k_feces × GutTCDD
FecesTCDD′ = k_feces × GutTCDD
cum_exposure′ = LiverTCDD
BloodProtein = MaxBloodProtein × LiverVolume × K_i_BloodProtein/(K_i_BloodProtein + LiverTCDD/LiverVolume)
BloodTCDD′ = k_absorption × GutTCCD/BodyWeight$^{0.75}$ − BloodTCDD × MuscleDiffusion × MuscleFlow/BloodVolume − BloodTCDD × FatDiffusion × FatFlow/BloodVolume − BloodTCDD × VisceraDiffusion × VisceraFlow/BloodVolume − BloodTCDD × LiverDiffusion × LiverFlow/BloodVolume + Muscle TCDD × MuscleDiffusion × MuscleFlow/(Muscle Volume × MusclePartition) + FatTCDD × FatDiffusion × FatFlow/(FatVolume × FatPartition) + VisceraTCDD × VisceraDiffusion × VisceraFlow/(VisceraVolume × VisceraPartition) + LiverTCDD × LiverDiffusion × LiverFlow/(LiverVolume × LiverPartition) − k_binding × BloodTCDD × BloodProtein + k_binding × K_BloodProtein × BloodVolume × BloodBound TCDD
BloodBoundTCDD′ = k_binding × BloodTCDD × BloodProtein − k_binding × K_BloodProtein × BloodVolume × BloodBoundTCDD
Muscle TCDD′ = BloodTCDD × MuscleDiffusion × MuscleFlow/BloodVolume − MuscleTCDD × MuscleDiffusion × MuscleFlow/(Muscle Volume × MusclePartition)
FatTCDD′ = BloodTCDD × FatDiffusion × FatFlow/BloodVolume − FatTCDD × FatDiffusion × FatFlow/(FatVolume × FatPartition)
VisceraTCDD′ = BloodTCDD × VisceraDiffusion × VisceraFlow/BloodVolume − VisceraTCDD × VisceraDiffusion × VisceraFlow/(VisceraVolume × VisceraPartition)
LiverTCDD′ = BloodTCDD × LiverDiffusion × LiverFlow/BloodVolume − LiverTCDD × LiverDiffusion × LiverFlow/(LiverVolume × LiverPartition) − k_metabolism × LiverTCDD/BodyWeight$^{0.3}$ − k_binding × LiverTCDD × LiverCYP1A2 + k_binding × K_TCDD × LiverVolume × LiverCYP1A2_TCDD − k_binding × LiverTCDD × LiverAhReceptor + k_binding × K_AhR × LiverVolume × LiverAhR_TCDD + k_proteolysis × (LiverAhR_TCDD + LiverCYP1A2_TCDD) − k_lysis × cum_exposure/(crit_exposure + cum_exposure) × LiverTCDD
LiverCYP1A2_TCDD′ = k_binding × LiverTCDD × LiverCYP1A2 − k_binding × K_TCDD × LiverVolume × LiverCYP1A2_TCDD

TCDD Metabolite Clearance

LiverMetabolite′ = k_metabolism × LiverTCDD/BodyWeight$^{0.3}$ − k_bile × LiverMetabolite + BloodMetabolite × LiverDiffusion × LiverFlow/BloodVolume − LiverMetabolite × LiverDiffusion × LiverFlow/(LiverVolume × LiverPartition)

APPENDIX 1: Continued

BloodMetabolite' = −BloodMetabolite × LIverDiffusion × LiverFlow/BloodVolume + LiverMetabolite × LiverDiffusion × LiverFlow/(LiverVolume × LiverPartition) − k_urine × BloodMetabolite

UrineMetabolite' = k_urine × BloodMetabolite

GutMetabolite' = k_bile × LiverMetabolite − k_feces × GutMetabolite

FecesMetabolite' = k_feces × GutMetabolite

Ah Receptor and P450 Induction

LiverAhR_TCDD' = k_binding × LiverTCDD × LiverAhReceptor − k_binding × K_AhR × LiverVolume × LiverAhR_TCDD − k_proteolysis × LiverAhR_TCDD

LiverAhR_Inducer' = k_binding × LiverVolume × Natural_Inducer × LiverAhReceptor − k_binding × K_AhR × LiverVolume × LiverAhR_Inducer − k_proteolysis × LiverAhR_Inducer

LiverAhReceptor' = V_AhRinduction × LiverVolume/(1 + (K_AhRinduction × LiverVolume)/LiverAhR_TCDD(t − lt)) + AhRexpression × LiverVolume − k_binding × LiverTCDD × LiverAhReceptor + k_binding × K_AhR × LiverVolume × LiverAhR_TCDD − k_binding × LiverVolume × Natural_Inducer × LiverAhReceptor + k_binding × K_AhR × LiverVolume × LiverAhR_Inducer − k_proteolysis × LiverAhReceptor

LiverCYP1A1' = V_CYP1A1induction × LiverVolume/(1 + (K_CYP1A1induction × LiverVolume)/(LiverAhR_TCDD(t − lt) + LiverAhR_Inducer(t − lt))) − k_proteolysis × LiverCYP1A1 − k_binding × LiverCYP1A1 × LiverP450Reductase + k_binding × K_Reductase × LiverVolume × LiverCYP1A1_R

LiverP450Reductase' = P450Red_expression × LiverVolume − k_proteolysis × LiverP450Reductase − k_binding × LiverCYP1A1 × LiverP450Reductase + k_binding × K_Reductase × LiverVolume × LiverCYP1A1_R − k_binding × LiverCYP1A2 × LiverP450Reductase + k_binding × K_Reductase × LiverVolume × LiverCYP1A2_R − k_binding × LiverCYP1A2_E2 × LiverP450Reductase + k_binding × K_Reductase × LiverVolume × LiverCYP1A2_R_E2

LiverCYP1A1_R' = k_binding × LiverCYP1A1 × LiverP450Reductase − k_binding × K_Reductase × LiverVolume × LiverCYP1A1_R

Estradiol Metabolism

LiverE2' = ConcBloodE2 × LiverFlow − LiverE2 × LiverFlow/LiverVolume − k_binding × LiverCYP1A2 × LiverE2 + k_binding × K_E2 × LiverVolume × LiverCYP1A2_E2 − k_binding × LiverCYP1A2_R × LiverE2 + k_binding × K_E2 × LiverVolume × LiverCYP1A2_R_E2 + k_proteolysis × LiverER_E2 − k_binding × LiverEReceptor × LiverE2 + k_binding × K_ER_E2 × Liver Volume × LiverER_E2

LiverCYP1A2' = V_CYP1A1induction × LiverVolume/(1 + (K_CYP1A2induction × LiverVolume)/LiverAhR_TCDD(t − lt)) + CYP1A2expression × LiverVolume − k_proteolysis × (LiverCYP1A2 + LiverCYP1A2_E2) − k_binding × LiverCYP1A2 × LiverP450Reductase + k_binding × K_Reductase × LiverVolume × LiverCYP1A2_R − k_binding × LiverCYP1A2 × LiverE2 + k_binding × K_E2 × LiverVolume × LiverCYP1A2_E2

LiverCYP1A2_R' = k_binding × LiverCYP1A2 × LiverP450Reductase − k_binding × K_Reductase × LiverVolume × LiverCYP1A2_R + V_E2H × LiverCYP1A2_R_E2

LiverCYP1A2_E2' = k_binding × LiverCYP1A2 × LiverE2 − k_binding × K_E2 × LiverVolume × LiverCYP1A2_E2 − k_binding × LiverCYP1A2_E2 × LiverP450Reductase + k_binding × K_Reductase × LiverVolume × LiverCYP1A2_R_E2

LiverCYP1A2_R_E2' = k_binding × LiverCYP1A2_E2 × LiverP450Reductase − k_binding × K_Reductase × LiverVolume × LiverCYP1A2_R_E2 + k_binding × LiverCYP1A2_R × LiverE2 − k_binding × K_E2 × LiverVolume × LiverCYP1A2_R_E2 − V_E2H × LiverCYP1A2_R_E2

LiverE2OH' = V_E2H × LiverCYP1A2_R_E2 − k_binding × LiverEReceptor × LiverE2OH + k_binding × K_ER_E2OH × LiverVolume × LiverER_E2OH − k_conjugation × LiverE2OH

APPENDIX 1: Continued

LiverEReceptor′ = V_ERinduction × LiverVolume/((1 + K_ERinduction × LiverVolume/(LiverER_E2(t − lt) + LiverER_E2OH(t − lt))) × (1 + LiverAhR_TCDD(t − lt)/(K_i_ERinduction × LiverVolume))) + ERexpression × LiverVolume − k_proteolysis × LiverEReceptor − k_binding × LiverEReceptor × LiverE2 + k_binding × K_ER_E2 × LiverVolume × LiverER_E2 − k_binding × LiverEReceptor × LiverE2OH + k_binding × K_ER_E2OH × LiverVolume × LiverER_E2OH

LiverER_E2′ = −k_binding × LiverEReceptor × LiverE2 + k_binding × K_ER_E2 × LiverVolume × LiverER_E2 − k_proteolysis × LiverER_E2

LiverER_E2OH′ = −k_binding × LiverEReceptor × LiverE2OH + k_binding × K_ER_E2OH × LiverVolume × LiverER_E2OH − k_proteolysis × LiverER_E2OH

EGF Receptor and TGF-α

LiverEGFReceptor′ = EGFRexpression × LiverVolume − k_endocytosis × LiverEGFReceptor

LiverTGF′ = V_TGFinduction × LiverVolume/(1 + K_TGFinduction × LiverVolume/(LiverAhR_TCDD(t − lt) × (1 + K_a_TGFinduction × LiverVolume/(LiverER_E2(t − lt) + LiverER_E2OH(t − lt))))) − k_proteolysis × LiverTGF − k_binding × LiverEGFReceptor × LiverTGF + k_binding × K_TGF × LiverVolume × LiverInternalEGFR

LiverInternalEGFR′ = k_binding × LiverEGFReceptor × LiverTGF − k_binding × K_TGF × LiverVolume × LiverInternalEGFR − k_endocytosis × LiverInternalEGFR

Initial Conditions

LiverAhReceptor = 0.017806 nmol (Sloop and Lucier, 1987)
LiverAhR_Inducer = 0.000674 nmol (determined from conservation of mass)
LiverCYP1A1 = 0.063174 nmol (Tritscher *et al.*, 1992)
LiverCYP1A1_R = 0.094902 nmol (determined from conservation of mass)
LiverCYP1A2 = 2.4199 nmol (Tritscher *et al.*, 1992)
LiverCYP1A2_R = 1.9799 nmol (determined from conservation of mass)
LiverP450Reductase = 0.602536 nmol (Miwa *et al.*, 1978)
LiverE2 = 0.00123 nmol (assumed at equilibrium with blood)
LiverEReceptor = 0.01495 nmol (Clark *et al.*, 1991)
LiverER_E2 = 0.02325 nmol (determined from conservation of mass)
LiverER_E2OH = 0.0066792 nmol (determined from conservation of mass)
LiverEGFReceptor = 0.022563 nmol (Sewall *et al.*, 1993)
(All other state variables start at zero)

APPENDIX 2

Physiological Parameters

frac_interstitium = 0.1
lt = 0.25 day (Sloop and Lucier, 1987) (delay between binding of liganded receptor to DNA and appearance of induced proteins in the liver cell)
ConcBloodE2 = 0.185 nmol/liter (Shiverick and Muther, 1983)
crit_exposure = 0.3 nmol

Production of Blood Proteins

MaxBloodProtein = 5 nmol/liter (determined from the data of Tritscher *et al.*, 1992)
K_i_BloodProtein = 0.1 nM (determined from the data of Tritscher *et al.*, 1992)
K_BloodProtein = 0.4 nM (determined from the data of Tritscher *et al.*, 1992)

APPENDIX 2: Continued

Receptor Binding Constants

K_AhR	= 0.27 nM (apparent K_d in biological materials; Poland and Knutson, 1982)
K_ER_E2	= 0.13 nM (Vickers *et al.*, 1987)
K_ER_E2OH	= 1.3 nM (Li *et al.*, 1985)
K_TGF	= 0.28 nM (Hudson *et al.*, 1985)
K_TCDD	= 30 nM (Poland *et al.*, 1989; Voorman and Aust, 1989)

Constitutive Expression Rates

AhRexpression	= 1.4553 nmol/liter/day (determined from conservation of mass)
ERexpression	= 0.67273 nmol/liter/day (determined from conservation of mass)
CYP1A2expression	= 213.4 nmol/liter/day (determined from conservation of mass)
P450Reductase_expression	= 47.45 nmol/liter/day (determined from conservation of mass)
EGFRexpression	= 0.69376 nmol/liter/day (determined from conservation of mass)

Endogenous Ligand of Ah Receptor

Natural_Inducer	= 0.0015 nmol/liter (determined from the data of Tritscher *et al.*, 1992; assumed same K_d as for TCDD)

Gene Induction Parameters

V_AhRinduction	= 7.2 nmol/liter/day (Lower bound of 4 nmol/liter/day; Sloop and Lucier, 1987)
K_AhRinduction	= 4 nM (Sloop and Lucier, 1987)
V_CYP1A1induction	= 3319 nmol/liter/day (determined from the data of Tritscher *et al.*, 1992)
K_CYP1A1induction	= 4.279 nM (determined from the data of Tritscher *et al.*, 1992)
V_CYP1A2induction	= 4197 nmol/liter/day (determined from the data of Tritscher *et al.*, 1992)
K_CYP1A2induction	= 7.458 nM (determined from the data of Tritscher *et al.*, 1992)
V_ERinduction	= 3.2488 nmol/liter/day (Romkes *et al.*, 1987)
K_ERinduction	= 0.35 nM (determined from the data of Clark *et al.*, 1991)
K_i_ERinduction	= 3.1 nM (determined from the data of Clark *et al.*, 1991)
V_TGFinduction	= 1.5 nmol/liter/day (determined from the data of Sewall *et al.*, 1993)
K_TGFinduction	= 9 nM (determined from the data of Sewall *et al.*, 1993)
K_a_TGFinduction	= 0.75 nM (determined from the data of Sewall *et al.*, 1993)

Metabolic Parameters

V_E2H	= 8496 day^{-1} (Graham *et al.*, 1988)
K_E2	= 9400 nM (Voorman and Aust, 1987)
K_Reductase	= 83.5 nM (Miwa *et al.*, 1987)

Rate Constants

k_absorption	= 4.8 $kg^{0.75}$/day (Leung *et al.*, 1990)
k_binding	= 10^5 $nmol^{-1}$ day^{-1} (value selected to ensure that binding reactions are always close to equilibrium)
k_proteolysis	= 0.693 day^{-1} (Lucier *et al.*, 1972)
k_endocytosis	= 0.271 day^{-1} (determined from the data of Sewall *et al.*, 1993)
k_metabolism	= 2.75 day^{-1}
k_urine	= 5.36 day^{-1} (Birnbaum *et al.*, 1980)
k_bile	= 3.81 day^{-1} (Birnbaum *et al.*, 1980)
k_feces	= 1.152 day^{-1} (Lutz *et al.*, 1977)
k_conjugation	= 56.693 day^{-1} (Lucier and McDaniel, 1977)
k_lysis	= 9.5 day^{-1}

APPENDIX 2: Continued

Partition and Diffusion Coefficients

FatPartition	= 350 (Leung *et al.*, 1990)
MusclePartition	= 40 (Leung *et al.*, 1990)
VisceraPartition	= 20 (Leung *et al.*, 1990)
LiverPartition	= 20 (Leung *et al.*, 1990)
FatDiffusion	= 0.1 (selected to fit the data of Abraham *et al.*, 1988)
MuscleDiffusion	= 0.5 (selected to fit the data of Abraham *et al.*, 1988)
VisceraDiffusion	= 0.5 (selected to fit the data of Abraham *et al.*, 1988)
LiverDiffusion	= 0.4 (selected to fit the data of Abraham *et al.*, 1988)

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APPENDIX B

Modeling Receptor-Mediated Processes with Dioxin: Implications for Pharmacokinetics and Risk Assessment

Melvin E. Andersen,[1,6] Jeremy J. Mills,[1] Michael L. Gargas,[1] Lorrene Kedderis,[2] Linda S. Birnbaum,[3] Diether Neubert,[4] and William F. Greenlee[5]

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Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD), a widespread polychlorinated aromatic hydrocarbon, caused tumors in the liver and other sites when administered chronically to rats at doses as low as 0.01 μg/kg/day. It functions in combination with a cellular protein, the *Ah* receptor, to alter gene regulation, and this resulting modulation of gene expression is believed to be obligatory for both dioxin toxicity and carcinogenicity. The U.S. EPA is reevaluating its dioxin risk assessment and, as part of this process, will be developing risk assessment approaches for chemicals, such as dioxin, whose toxicity is receptor-mediated. This paper describes a receptor-mediated physiologically based pharmacokinetic (PB-PK) model for the tissue distribution and enzyme-inducing properties of dioxin and discusses the potential role of these models in a biologically motivated risk assessment. In this model, ternary interactions among the *Ah* receptor, dioxin, and DNA binding sites lead to enhanced production of specific hepatic proteins. The model was used to examine the tissue disposition of dioxin and the induction of both a dioxin-binding protein (presumably, cytochrome P4501A2), and cytochrome P4501A1. Tumor promotion correlated more closely with predicted induction of P4501A1 than with induction of hepatic binding proteins. Although increased induction of these proteins is not expected to be causally related to tumor formation, these physiological dosimetry and gene-induction response models will be important for biologically motivated dioxin risk assessments in determining both target tissue dose of dioxin and gene products and in examining the relationship between these gene products and the cellular events more directly involved in tumor promotion.

KEY WORDS: PB-PK modeling; dioxin; gene regulation; cytochrome P450; risk assessment; pharmacokinetics; pharmacodynamics.

1. INTRODUCTION

Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD), a carcinogen[1,2] and tumor promoter[3] in rodents, also causes immunotoxic,[4] reproductive,[5] and teratogenic[6] effects. The present U.S. EPA cancer risk assessment, based on the incidence of liver tumors in female rats dosed with dioxin for 2 years,[1] utilizes the standard linearized multistage (LMS) approach, with a one in a million risk level, to derive a virtually safe dose of 6 femtograms dioxin/kg body weight per day.[7] Other Western countries treat dioxin as a tumor promoter, with a threshold for its effects, and arrive at acceptable or tolerable daily intake values up to 1000-fold higher than that used by the United States.[8]

All of the toxic effects of dioxin noted above are believed to be dependent on the interaction of dioxin with a specific cellular protein, the *Ah* (*a*ryl *h*ydrocarbon) receptor. The *Ah* receptor-dioxin complex binds to

[1] Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, North Carolina 27709.
[2] Curriculum in Toxicology, Center for Environmental Medicine, University of North Carolina, Chapel Hill, North Carolina 27599.
[3] Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.
[4] Institute of Toxicology and Embryopharmacology, Free University Berlin, Garystr 5, D-1000 Berlin 33, Germany.
[5] Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907-7880.
[6] To whom all correspondence should be addressed.

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specific sites on DNA modifying regulation of various genes, some of which can alter cell growth and differentiation, while others affect metabolic processes (Fig. 1). Due to the obligate role of the *Ah* receptor in its toxic effects, dioxin has been referred to as a "receptor-mediated" carcinogen. Dioxin is only one of many polyhalogenated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls that alter cell growth characteristics *via* interactions with the *Ah* receptor. The EPA is reviewing the current dioxin risk assessment with the stated intention of developing a generic, biologically realistic approach to risk assessment for these "receptor-mediated" agents.[9] With improved knowledge of the biological basis of the action of dioxin at the molecular level, it may well prove possible to reconcile the present disparity among different countries.

Dioxin demonstrates dose-dependent kinetics: as the administered dose increases, a larger proportion of the total dose is found in the liver.[10] Among the hepatic proteins induced by dioxin is a cytochrome, P4501A2, which has a high affinity for dioxin.[11,12] It appears likely that dose-dependent hepatic sequestration is related to induction of P4501A2, but other proteins may also be involved. Any comprehensive model of dioxin pharmacokinetics must include the induction of these dioxin-binding proteins, mediated by the interaction of a dioxin-*Ah*-receptor complex with specific binding sites on DNA. Leung and co-workers developed a physiologically based pharmacokinetic (PB-PK) model for dioxin and congeners in mice[13] and rats.[14] Their model included induction of a single hepatic binding species occurring in direct proportion to the fractional occupancy of the available *Ah* receptor by dioxin, but did not include direct interactions of the *Ah*-TCDD complex with DNA. In mice, dioxin pretreatment increased metabolic clearance of dioxin-like analogs.[15]

This paper extends the earlier PB-PK models by including induction of binding proteins/enzymes and of dioxin metabolism in response to ternary interactions of dioxin, the *Ah* receptor, and DNA binding sites and analysis of repeated dose exposure situations. This physiological model for dioxin disposition and enzyme induction is discussed in relation to its potential role for estimating dosimetry and gene regulation in receptor-based risk assessment strategies for dioxin.

2. PB-PK MODEL

2.1. Model Structure

The dioxin PB-PK model (Fig. 2) consists of five compartments—liver, fat, slowly perfused tissues (e.g.,

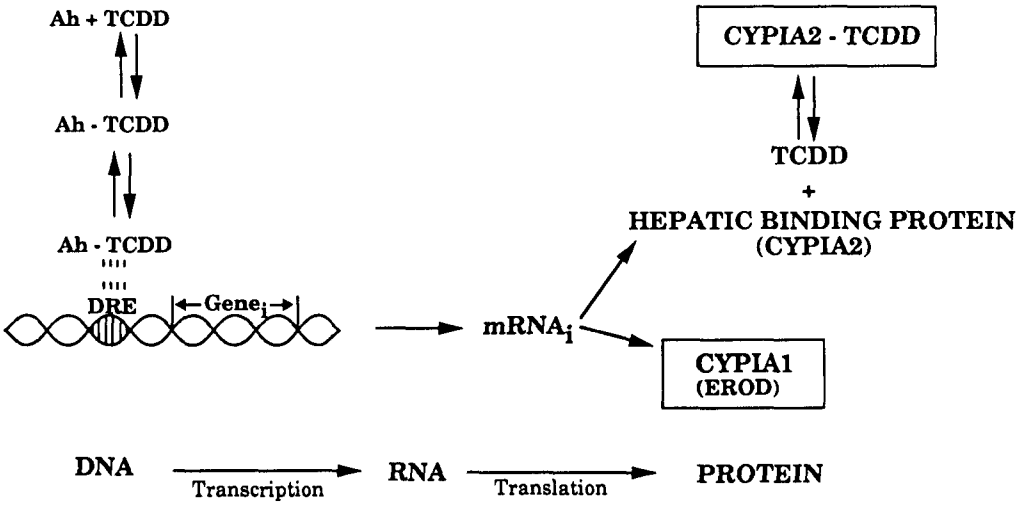

Fig. 1. Schematic of the molecular mechanisms of action of dioxin. Dioxin binds to a cellular protein, the *Ah* receptor, and the dioxin-receptor complex interacts with DNA sites in regulatory regions of certain genes. Interaction with these sites, called dioxin-responsive elements (DREs), leads to changes in rates of gene transcription (i.e., the rate at which mRNA is produced from these genes). The mRNAs serve as templates for protein synthesis. Changes in cellular mRNA levels lead to altered protein concentrations for cytochrome P4501A1, hepatic, dioxin-binding proteins, metabolizing enzymes, and growth factors. The change in concentration of certain of these proteins is believed to be associated with the various toxic effects of dioxin.

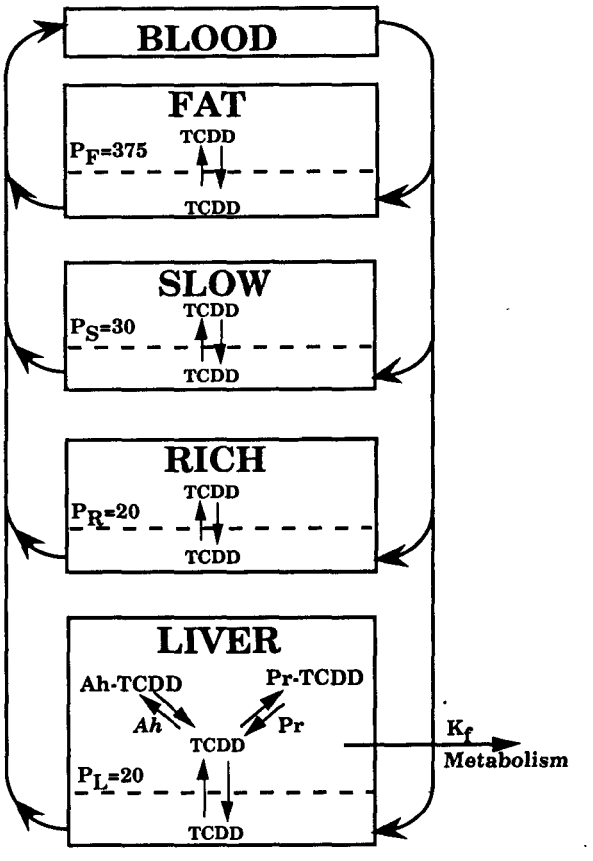

Fig. 2. The PB-PK model for dioxin. A five-compartment diffusion-limited model was developed with metabolism and protein binding included in the liver. Uptake after subcutaneous administration was described as a first-order process with the chemical appearing in mixed venous blood. Symbol definitions are in Table I.

muscle, skin, etc.), richly perfused tissues (e.g., kidney, brain, etc.), and blood. The relevant mass-balance differential equations appear in the Appendix and the terms are defined in Table I. Each of the four tissue compartments (denoted by subscript $_t$) has both a specified blood flow (Q_t), tissue compartment volume (V_t), and a tissue blood volume (V_{tb}). The tissue blood volumes were estimated from Bischoff and Brown.[16] Movement of chemical from the tissue blood into the tissue is modeled to be proportional to a permeation coefficient-surface area cross-product (PA) for the tissue ($_t$). Tissue uptake is diffusion-limited when $PA_t < Q_t$. Each tissue has a specified volume, blood flow, PA product, and partition coefficient (P_t). In the Leung *et al.* models,[13,14] tissues had no specified blood volume and the tissue/tissue blood aggregates were described as flow-limited compartments. In addition, there was binding in the blood com-

Table I. Parameters in the Physiological Dosimetry Model for Dioxin[a]

Model parameters body weight (kg)	Abbreviations bw	Wistar rat[b] 0.215
Volumes[c] (liters)		
Liver	V_l	0.0375 × bw
Fat	V_f	0.07 × bw
Richley perfused (viscera)	V_r	0.0525 × bw
Slowly perfused (muscle/skin)	V_s	0.75 × bw
Blood	V_b	0.05 × bw
Tissue blood volumes		
Liver	V_{lb}	0.01 × V_l
Fat	V_{fb}	0.05 × V_f
Richly perfused	V_{rb}	0.01 × V_r
Slowly perfused	V_{sb}	0.05 × V_s
Cardiac output (liters/hr)	Q_c	4.4
Tissue blood flow (% Q_c)		
Liver (portal and arterial)	Q_l	0.25
Richly perfused	Q_r	0.51
Fat	Q_f	0.09
Slowly perfused	Q_s	0.15
Diffusional tissue clearance (liters/hr)		
Liver	PA_l	0.5 × Q_l
Fat	PA_f	0.2 × Q_f
Richly perfused	PA_r	0.5 × Q_r
Slowly perfused	PA_s	0.5 × Q_s
Metabolic constants		
Metabolism (hr^{-1})	kfc	1.65
Induction (fold over basal rate)	Fold	1.00
Partition coefficients		
Liver/blood	P_l	20
Fat/blood	P_f	375
Richly perfused/blood	P_r	20
Slowly perfused/blood	P_s	30
Protien binding		
Ah maximum (pmol/liver)	BM_1	3.75
Ah affinity (pM)	KB_1	35
1A2 basal level (nmol/liver)	$BM2_0$	10
1A2 maximum (nmol/liver)	$BM2_1$	85
1A2 affinity (nM)	KB_2	6.5
1A2 Hill term	n	1.0
1A2 Hill binding constant (pM)	K_d	50
1A1 Hill term	n_1	2.3
1A1 Hill binding constant (pM)	K_{d1}	180
1A1 degradation rate constant (hr^{-1})	k_1	0.035
1A1 synthesis-basal rate (units/hr)	K_0	0.7
1A1 maximum induction (fold)	K_{0max}	50

[a] Deviations from this base parameter set are noted in the legends to each figure.

[b] Parameters are for the 215 g rat at the start of the dose–response study in Fig. 3.

[c] Tissue volumes are given as liters and referenced to tissue as a proportion of the total body weight, assuming unit density (1 kg = 1 L)

partment which effectively decreased the rate of tissue uptake. Our revised model, because of the diffusion-limited tissue compartments, does not require blood binding to match tissue uptake time-course behavior.

Initial estimates of partition coefficients were obtained from Leung *et al.*[14] and were adjusted in fitting the data sets here. As expected, the fat has the highest partition coefficient due to the highly lipophilic nature of dioxin. In addition, metabolism and protein binding are included in the liver, where both the *Ah* receptor and the inducible binding protein act to sequester dioxin *via* capacity-limited binding processes. The binding protein was assumed to be cytochrome P4501A2. The concentrations of P4501A2 in naive and induced rats differ by about 10-fold.[17] The differential equation for the liver (Eq. 5) is solved for the total amount of dioxin in liver, which is related to partitioned dioxin, dioxin bound to the *Ah* receptor, and dioxin bound to P4501A2 (Eq. 6). This conservation equation is used to solve for the free dioxin in the liver tissue, C_{lf}, which is then utilized in calculating the concentration of the *Ah* receptor-dioxin complex available for inducing cytochrome P4501A2 and cytochrome P4501A1 activity. This latter protein, an oxidative microsomal enzyme induced by dioxin, is frequently used as a marker of *Ah* receptor-mediated protein induction. All these binding interactions are described by simple, reversible equilibrium relationships. This approach is valid as long as the rate constants for association/dissociation are large (i.e., the processes are rapid). This description, like all models, is really a simplification of the more complex series of biological events. The model can readily be extended to include new information on these biological associations as it becomes available.

2.2. Enzyme/Protein Induction

The induction process is described by assuming that the *Ah*-TCDD complex is formed based on *Ah* receptor-binding parameters (BM_1 and KB_1) and free dioxin in the liver (C_{lf}). This complex then binds to unspecified sites on DNA with an affinity, K_d. Since the binding to DNA does not directly alter the *Ah*-TCDD concentration in this present model configuration, it is tacitly assumed that the DNA sites are present at much lower concentrations than the *Ah*-TCDD complex. The induction is modeled with a Hill plot binding relationship (Eq. 6), where n provides a measure of interaction for multiple *Ah*-TCDD complex binding sites. The value of n is estimated by comparison of model simulations to data on the dose-dependence of liver sequestration of dioxin or on the induction of P4501A1 activity (Figs. 3 and 4). Basal levels of P4501A1 are described by a zero-order production rate (K_0) and a first-order elimination rate constant (k_1). The induction process increases the production rate as specified by the Hill relationship and the maximum observable enhancement (K_{0max}) (Eq. 7). Data on the time course of cytochrome P4501A1 from Abraham *et al.*[10] were used to estimate the first-order degradation rate constant (k_1) for P4501A1 degradation.

Induction of hepatic binding capacity was also mod-

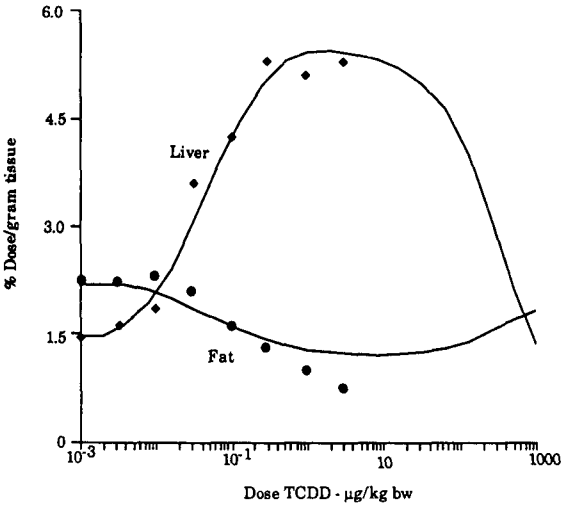

Fig. 3. Dose-dependence of dioxin tissue disposition in female Wistar rats 7 days following single subcutaneous doses. Tissue concentrations from Abraham *et al.*[10] are expressed normalized to dose, as %dose/tissue. Model parameters are given in Table I.

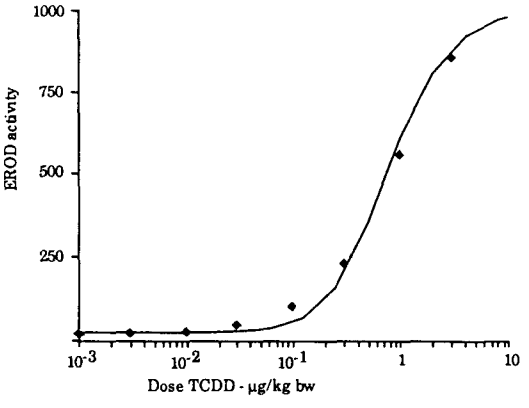

Fig. 4. Dose-dependence of cytochrome P4501A1 induction in female rats 7 days following single subcutaneous doses. Data from Abraham *et al.*[10] for activity of EROD (Ethoxyresorufin-O-deethylase) in pmoles resorufin formed/min/mg protein.

eled as if it were instantaneously altered by the *Ah* receptor-dioxin-DNA interactions (Eq. 8). As data becomes available for the time course of P4501A2 induction, synthesis and degradation rate constants for this protein can also be directly included in this description. For both cytochrome P4501A1 and 1A2 induction, it was assumed that *Ah*-TCDD complex formation was equivalent (similar Kb_1 and BM_1 values), but that the n value and K_d for the two responses differed (see Table I). Our model also allows an induction of dioxin metabolism following dioxin treatment. However, in contrast to the mouse, induction of metabolism in the rat, if present at all, was negligible. The constants employed assume at most only a doubling of the metabolic rate.

2.3. Data Analysis

The data used for this analysis were from two previously published studies with Wistar rats. The first study,[10] with female rats, provides both dose–response characterization of liver concentrations and of liver P4501A1 activity and time-course characterization of dioxin tissue concentrations and enzyme activities. The second study[18] examined liver and fat concentrations in male Wistar rats dosed weekly for periods up to 6 months.

Model simulations were performed using ACSL (Advanced Continuous Simulation Language, Mitchell and Gauthier Assoc., Concord, Massachusetts).

3. RESULTS

3.1. Dose–Response

In this study[10] rats received a single subcutaneous dose of dioxin and were killed 7 days later. The disposition of dioxin in liver and fat was highly dose-dependent in the concentration range between 1 ng/kg and 10,000 ng/kg (Fig. 3). Normalized concentrations (% dose/g tissue) would be horizontal lines if disposition were dose-independent. The curvature appears to be due to the induction of a dioxin-binding protein, presumably cytochrome P4501A2. The smooth curves were obtained with the PB-PK model based on the parameters in Table I. For induction of the binding protein, n and Kd were estimated by fitting the data from Wistar rats[10] and were, respectively, 1.0 and 50 pM. Using measured concentration estimates of basal and induced P4501A2,[17] the affinity of the binding protein ($KB2$) was estimated from the curve fitting to be 6.5 nM. A value of n close to 1.0 suggests little interaction among dioxin responsive DNA binding sites involved in expression of this particular gene. The complex behavior above 10 μg/kg bw is commented on more fully in the Discussion.

The dose–response curve for cytochrome P4501A1 induction (Fig. 4) was described in a similar manner, but required a larger value for n_1 (2.3) to fit the data, indicating possible interactions among DNA binding sites for the *Ah* receptor-TCDD complex with this gene. The half-maximal induction response for P4501A1 occurred at about a 10-fold higher dose than the half-maximal response of the binding protein (Kd_1 = 180 pM).

The PB-PK model configured for these dose–response curves was also used to examine time-course elimination/induction after a single dose of 300 ng/kg (Figs. 5 and 6). Analysis of these results were especially useful for setting the rate constant for metabolism of dioxin and the PA_t cross-products (i.e., the diffusion limitations) for liver and fat. The simulation of these curves requires inclusion of time-dependent growth parameters over the 100 days of the experiment. Growth rates and volumes of fat were estimated from growth curves for these rats available from suppliers.

3.2. Repeated Dosing

The primary health concerns with dioxin are associated with repeated low or chronic exposures. We analyzed data from a study in which liver and fat

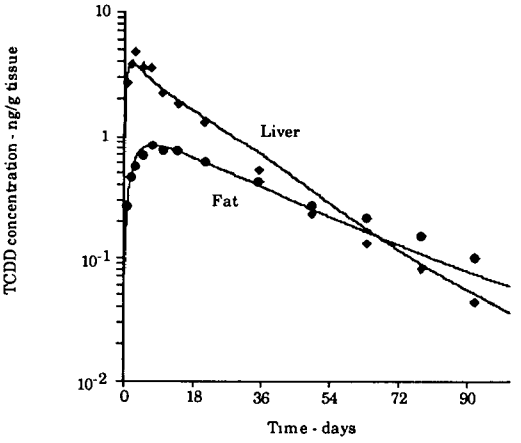

Fig. 5. Time course of liver and fat tissue dioxin concentrations following a single subcutaneous dose of 300 ng/kg in female Wistar rats. Concentrations are expressed as ng/g tissue, and the data are from Abraham *et al.*[10] Parameters used were the same as in Figs. 3 and 4; however, growth of the rat and fat compartments were also included.

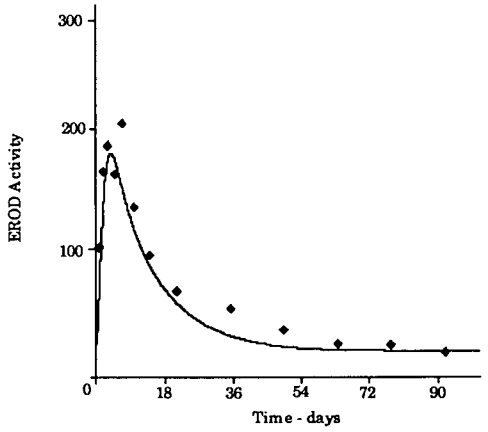

Fig. 6. Time course of cytochrome P4501A1 activity following a single dose of 300 ng dioxin/kg in female Wistar rats. Data are from Abraham *et al.*[10] and parameters as in Fig. 5.

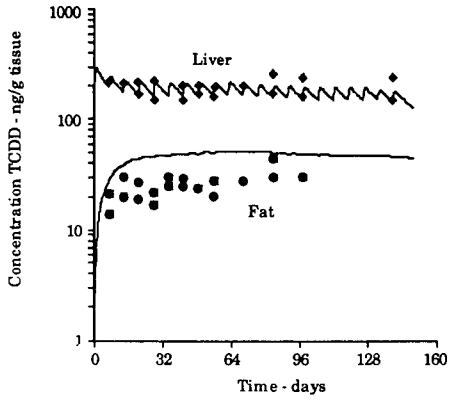

Fig. 7. Time course liver and fat concentrations in rats dosed weekly with 5μg dioxin/kg starting 7 days after a loading dose of 25 μg/kg. These data, expressed as ng/g tissue, are from Krowke *et al.* [18] The model parameters were as specified in other figures with several changes: P_f, P_s, P_{afc}, and *kfc* were, respectively 250, 50, 0.1 and 1.5. These contrast to values of 375, 30, 0.20, and 1.65.

concentrations were determined in male rats for up to 6 months during weekly dosing with dioxin.[18] This study (Fig. 7) used a loading dose (25 μg/kg) followed by a fivefold smaller dose (5 μg/kg) every week. The simulations for this dosing scenario were conducted with model parameters very similar to those used in the single-dose exposures (Figs. 3–6). Small differences in fat and slowly perfused tissue compartment parameters are noted in the figure legends.

3.3. Correlation with Responses

The present EPA dioxin risk assessment is based on liver tumors in female rats.[1] Liver tumors are also increased in female and male mice.[2] Liver initiation-promotion studies[3,19,20] demonstrate that dioxin is a promoter and that the dose–response curve for its promoting action on altered hepatic foci closely follows the dose–response curve of its hepatocarcinogenic activity (Table II). Both of these dose–effect relationships are very steep.

Based on our preliminary repeated-dose PB-PK model for these Wistar rats, several measures of dose can be calculated for comparison with the promotional efficacy and carcinogenicity of dioxin in Sprague-Dawley rats (Table II). These include integrated total liver dioxin concentration during the treatment period, or integrated free liver dioxin concentration. In addition, measures of tissue dose related to enhanced expression of cytochrome P4501A1 and hepatic binding proteins can be calculated for the duration of the subchronic exposure. The measures of tissue dose associated with en-

Table II. Relationship Between Hepatic Exposure to Dioxin, Hepatic Protein Induction, and Hepatic Toxicity During Subchronic Exposures

Dose[a]	Altered foci[b] volume	Tumors[c] (liver)	AUCL-free[d] C_{lf}	AUCL-total[e] C_l	AU-1A2[f] ($BM2_l$)	AU-1A1[g] (IND)
Control	0.8	1/86	0	0	0	0
0.0001	0.2	—	6.7×10^{-1}	3.5×10^{1}	7.0×10^{3}	2.3×10^{1}
0.001	0.3	0/50	6.4×10^{0}	5.9×10^{2}	5.7×10^{4}	3.9×10^{3}
0.01	0.7	2/50	5.3×10^{1}	1.1×10^{4}	2.1×10^{5}	2.7×10^{5}
0.1	2.8	11/49	4.2×10^{2}	1.2×10^{5}	3.2×10^{5}	2.0×10^{6}
1	—	—	3.8×10^{3}	1.0×10^{6}	3.5×10^{5}	3.0×10^{6}
10	—	—	3.8×10^{4}	5.4×10^{6}	3.5×10^{5}	3.1×10^{6}

[a] Biweekly doses used; dose expressed as average dose per day in μg/kg.

[b] Volume as percent of liver occupied by altered cells, from Pitot *et al.*[18]

[c] Liver tumors observed in female rats in the bioassay study of Kociba *et al.*[1]

[d] Total area under the curve for free dioxin in the liver, units are nmoles-hr/liters.

[e] Total area under the curve for total dioxin in the liver, units are nmoles-hr/liters.

[f] Integrated exposure to induced levels of binding protein; units are nmoles-hr/liter; and the calculation integrates the third term on the right side of Eq. (6).

[g] Integrated exposure to induced levels of cytochrome P4501A1; units are enzyme unit-hr; and the calculation only considers the enhanced level of activity due to induction.

hanced gene expression (assumed to be related to P4501A1 activity or the sequestration of dioxin in liver by cytochrome P4501A2) are the integrated level of these gene products (as protein concentration of 1A2, or activity of 1A1) over time. Enhanced expression refers to the increase over any basal levels of expression of these gene products. This correlative approach does not assume that there should be a direct relationship between induction of these cytochromes and the promotional action of dioxin at all dose levels.

The tumor promotional response of dioxin in the rat liver is most closely correlated with the integrated expression of the P4501A1 gene (Table II) under these exposure conditions. For instance, the responses (columns 2 and 3) increase rapidly with increasing dose between 0.01 and 0.1 μg/kg/day. The integrated level of cytochrome P4501A1 increases by a factor of about 10 in this range, while the integrated amounts of binding protein (P4501A2), whose induction is already nearly saturated by 0.01 μg/kg/day, only increases by 50% in this region of dose. Dioxin concentrations increase in this dose range, but dioxin itself is not believed to be responsible for toxic effects.

4. DISCUSSION

4.1. Pharmacokinetics

The half-life of dioxin in male rats was initially reported to be 20–30 days.[21] These early studies were conducted at high, inducing doses; elimination curves were obtained for only about two half-lives; and analysis relied on chemical detection of dioxin in tissues. The half-lives in hamsters (10–15 days) and guinea pigs (30–40 days) were also estimated from time course data with a simple one-compartment model for dioxin kinetics but used radiochemical detection of labeled dioxin.[22,23] These various studies were relatively insensitive to the dose-dependent effects apparent only when disposition is examined at much lower doses (Fig. 3) and most apparent when both fat and liver concentrations are measured. The alteration in the ratio of liver-to-fat concentration ratio is the most sensitive marker for this dose-dependence (compare Figs. 3 and 8b). These low dose effects can now be readily evaluated by use of high specific activity ^{3}H-labeled dioxin. Single doses of 1 ng/kg caused minimal induction of P4501A1 (Fig. 4) or of the dioxin binding protein (Fig. 3). Protein induction, however, becomes significant at a dose of 30–50 ng/kg dose and

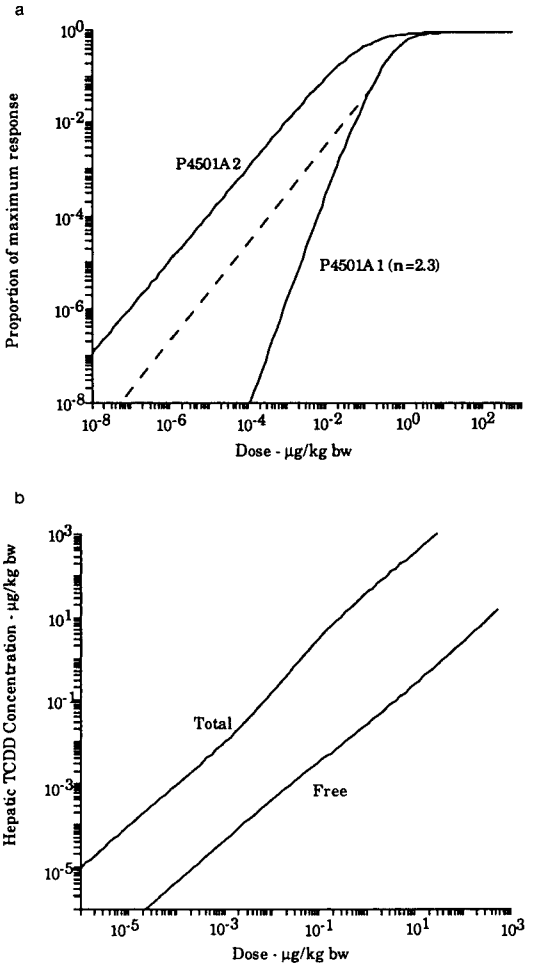

Fig. 8. Low-dose extrapolation of measures of tissue dose for dioxin in subchronic exposures. (A) Dose measures related to induction of cytochrome P4501A1 and the liver binding protein. The steeper line is for enhanced P4501A1 which has a cooperativity term of 2.3. Two extrapolations are depicted with cytochrome P4501A1; the case of a Hill coefficient of 2.3 throughout the dose range, and for the case of a linear relationship below several percent response (dashed line). the latter is more biologically realistic. (B) Dose measures for total liver dioxin and for free dioxin in liver. The total increases in a greater than linear fashion with dose in the region where induction of the binding protein occurs. In that same dose region the tissue dose of free dioxin does not increase in direct proportion to exposure level. These calculations used the model applied to data in Fig. 7 and calculated exposures for a 6-month period.

markedly alters dioxin disposition. Simple linear compartmental analyses of dioxin pharmacokinetics have completely ignored dose-dependent protein binding.

PB-PK models have been reported for polychlorinated biphenyls,[24] 2,3,7,8-tetrachlorodibenzofuran,[25]

and, in a preliminary form, for dioxin itself[26]. With the exception of the work by Leung and colleagues,[13,14] none of these descriptions have included specific hepatic binding. In all these other models, chemicals were distributed simply by nonspecific, dose-invariant partitioning and focused on behavior at higher doses with substantial induction of binding proteins. Because of the limited dioxin-binding capacity of the liver, even in fully induced animals, the liver/fat concentration ratio reaches a maximum and is predicted to decrease at higher body burdens (Fig. 3), where hepatic binding to induced proteins approaches saturation. While this region of dose was not examined by Abraham *et al.*,[10] the time course of elimination has been examined after a single dose of 600 μg/kg in hamsters, a species which is more resistant to the acute toxic effects of dioxin than are rats. In this study, the liver/fat ratio increased from 1.0–2.7 as the body burden fell from 600 to 100 μg/kg[22], consistent with the predictions in Fig. 3 for the dose range above 10 μg/kg bw.

Several people ingested relatively large amounts of dioxin-like polychlorinated dibenzofurans in the Yusho and Yu Cheng poisoning incidents in Japan and Taiwan, respectively. In several instances, liver and fat samples were obtained at autopsy, and the proportion of dose in the liver was found to be dependent on the total body burden. The % dose/liver increased from about 5 at ambient exposure levels to over 60 at a body burden of the furans toxicologically equivalent to a burden of 3–5 μg dioxin/kg body weight. The body burden for half maximal sequestration in the liver in people was estimated by Carrier *et al.*[27] to be 0.5–1.5 μg dioxin/kg body weight. Thus, this dose-dependent hepatic distribution is common both to rats and people with very similar body burdens required for half-maximal sequestration. The liver/fat concentration ratio, however, varies for different isomers. Higher chlorinated isomers tend to have ratios higher than for TCDD itself. The reasons for these differences are unknown; however, one suggestion derived from modeling efforts is that KB_2, the dioxin-binding protein dissociation constant, is lower for the higher-chlorinated analogs (i.e., they have higher affinities for the binding proteins than does dioxin itself).

4.2. Protein Binding/Induction

The challenge in providing a biologically realistic PB-PK model for dioxin is the need not only to account for the determinants of disposition (i.e., tissue partitioning, biotransformation rates, and protein binding constants), but also to describe the pharmacodynamic events related to the induction of specific dioxin-binding proteins in the liver. Knowledge of the molecular mechanisms of the interactions of the dioxin-*Ah* receptor complex with regulatory regions of specific genes is growing rapidly[28,29], and shows clearly a role for the ternary dioxin-*Ah* receptor-DNA complexes in regulating gene transcription. Our present model of disposition is based on a ternary complex being obligatory for the pharmacodynamic activity of dioxin at the genomic level. The description of these interactions requires estimates of binding constants between the *Ah* receptor and dioxin and between the *Ah* receptor-dioxin complex and sites on DNA. The ternary interactions with DNA are believed to enhance transcription of mRNA leading eventually to increased amounts of specific dioxin-binding proteins, particularly cytochrome P4501A2.

The binding maximum (*BM*1) and binding affinity (*KB*1) for TCDD binding to the *Ah* receptor have been previously reported. The values used in our model differ significantly: our estimate of *BM*1 is only about 10% of the value determined by Gasiewicz and Rucci[30] and our *KB*1 is also much lower than the value reported by Bradfield and Poland.[31] The discrepancy in *KB*1 is due simply to our decision to reference binding to the free dioxin in the liver. If we had used total partitioned dioxin, our number would increase by a factor of 20 and be more similar to the *in vitro* value.[31] However, the lower *BM*1 value represents a more fundamental discrepancy between *in vitro* measurements and *in vivo* pharmacokinetics.

In the present study, the binding parameters of the *Ah* receptor were largely estimated from the tissue concentrations of TCDD at noninducing levels, shown in Fig. 3. The liver concentration at doses below 0.01 μg/kg is especially sensitive to BM_1, the *Ah*-binding maximum. In addition, the dose at which hepatic sequestration is half-maximal is extremely sensitive to KB_1, the *Ah*-binding affinity. If *BM*1 were increased to the literature value, the liver concentration of TCDD in the low dose region (*i.e.*, noninduced animals) would be greatly overestimated. One possibility to explain this discrepancy is that only a relatively small proportion of the available receptors participate in binding dioxin and maintaining induction at physiologically realistic concentrations of TCDD *in vivo*. In mouse hepatoma cell lines, the total receptor concentration decreases after exposure to dioxin.[32]

The other important binding constant *Kd*, for the DNA interactions, also affects the placement of the induction curve, whether for 1A2 or 1A1, along the dose axis. The Hill-type coefficients control the steepness of the induction response. With the two responses exam-

ined, induction of liver sequestration (i.e., 1A2) shows higher affinity (lower *Kd* value) but lower cooperativity (smaller *n* value) than does induction of 1A1. This apparent cooperativity with 1A1 ($n_1 = 2.3$) is consistent with the observation that there are at least four dioxin responsive elements in the regulatory region of this gene.[28]

The PB-PK model structure developed here assumes that a single type of *Ah* receptor-dioxin complex interacts with DNA binding sites of variable affinity to regulate different genes. More complex models with different *Ah* receptor-dioxin complexes (due to other protein interactions, for instance) might lead to different conclusions about cooperativity in these two responses, but such models do not seem warranted by available biological data at this time.

4.3. Pharmacokinetics of the *Ah* Receptor

The biological activity of dioxin is a consequence of both delivery of dioxin to target cells and the dynamics of the processes that regulate *Ah* receptor concentration and *Ah* receptor binding characteristics. The present model extends our understanding of the biological determinants of dioxin action *in vivo*; however, it does not account for time variant changes in the receptor. In completely induced animals, only a relatively small fraction of total basal receptor binds to nuclear sites, even with complete induction of P4501A1 activity.[33] Furthermore, in mouse hepatoma cells *in vitro*, the total cellular receptor concentration decreases after treatment of the cells with dioxin.[32] In contrast, there is an apparent increase in *Ah* receptor in the rat liver following TCDD treatment.[34] These complexities in *Ah*-receptor kinetics should have minimal effects on our analysis, which focuses on longer-term behaviors, after 7 days or over the 100-day excretion period. New experimental techniques with appropriate antibodies[35] should soon be available for measuring the total amount of cellular *Ah* receptor. With these techniques, *Ah* receptor kinetics can be more fully investigated by examining total amount of receptor, receptor distribution, tissue dioxin, mRNA, and protein for P4501A2 over the first several hours to days after intravenous administration of dioxin. Such studies will improve both our appreciation of the pharmacokinetics/pharmacodynamics of the *Ah* receptor, and of the dosimetry of dioxin itself. It bears repetition that the determinants of dioxin pharmacokinetics cannot be examined in the absence of additional information. The pharmacokinetics of dioxin, of the *Ah* receptor, and of hepatic binding proteins are inextricably coupled.[36] Continued progress in physiological modeling of dioxin pharmacokinetics now relies on the progress in uncovering the biology of the *Ah* receptor,[37] especially regarding its ability to affect the expression of dioxin-binding proteins.

The current PB-PK model greatly simplifies the sequence of processes involved in activation of the receptor by dioxin. Normally, the receptor is complexed with heat shock protein(s) 90, which probably dissociate after the receptor binds dioxin.[38,39] Another protein species is then required for the interaction of the complex with DNA sites.[40] In the future, it will be necessary to model these events if they are found to be critical control elements for gene regulation; however, they are not understood in sufficient detail to justify their inclusion in the dosimetry model at this time.

4.4. Risk Assessment Implications

Greenlee *et al.*,[29] in a recent perspective on biologically based risk assessment for dioxin, outlined the biological steps involved in receptor-mediated growth modulatory effects. They are recognition, transduction, and response (Fig. 1). Our PB-PK model includes elements of recognition (binding of dioxin to the *Ah* receptor), transduction (binding of the complex to DNA), and response (the changes in rate of transcription/translation of P4501A1 and the binding protein). The responses of these two proteins are not thought to be causally linked to the adverse effects of dioxin. Specific mitogenic and mito-inhibitory cellular processes altered by dioxin treatment are more likely causally related to toxicity.[41,42] Even if we had modeled regulation of growth factor proteins, it is unclear what specific role they play, singly or in combination, in cell replication, cell differentiation, or toxicity. It will be of interest to examine the kinetic characteristics of induction (or repression) of these other proteins to see if they are controlled in a manner similar to P4501A1 or the sequestering protein. Since data on modulation of growth regulatory genes is not yet available, we have correlated various tissue dose measures from the present model with the promotional efficacy of dioxin. Of the several measures of tissue dose examined (Table II), the integrated exposure to P4501A1 is most closely correlated with promotion and with hepatic tumors. However, there is no expectation of causality between tumor responses and these induced proteins, and this correlation should be regarded cautiously.

In using the integrated P4501A1 exposure, the low dose extrapolation slope approaches the value of the Hill

coefficient ($n_1 = 2.3$), reflecting the cooperativity which appears to exist for induction of this gene by the ternary TCDD-*Ah* receptor-DNA interactions (Fig. 8a). The differences in the low dose extrapolation with measures of tissue dose related to protein induction (Fig. 8b) is related both to their respective slopes (n values) and the ease of induction by dioxin (the values of Kd and Kd_1). The mathematical formulation of these cooperativity interactions is based on a $(n+1)$ order interaction. Such interactions are highly unlikely since the rate of these association processes fall sharply with decreasing ligand concentration. Normally, cooperative relationships arise due to enhanced affinity for a second ligand molecule after the binding of the first ligand. In this way, all events involved are bimolecular. The Hill equation is simply a convenient way to treat this more complex series of events. With these multiple bimolecular steps, the low dose induction behavior should have a slope of 1 and be characterized by the binding parameters of the first ligand. Dose measures related to free or total dioxin in liver are also complexly related to administered dose; neither curve is strictly linear with dose and the departures from linearity are in opposite directions.

Appropriate measures of tissue dose for risk assessment must focus on specific cellular events, such as cell proliferation rates with developing preneoplastic foci in the livers of dioxin-treated rodents. Among the important ongoing efforts to improve the biological basis of dioxin risk assessment are studies analyzing regulation of dioxin-responsive growth regulatory genes in liver[43] and the effect of these genes on replication and focal growth. Eventually, biological response models will have to predict the relationship between replication or differentiation and the tissue exposure to these growth factors. Only when this level of resolution is achieved will it be possible to more fully justify any particular approach to low-dose extrapolation with dioxin.

The basic behavior of dose-dependent hepatic sequestration is a characteristic of many congeners of dioxin[44] and is observed in several species including people.[45] This basic PB-PK model structure appears to be applicable to many chemicals that act *via* interactions with the *Ah* receptor and should prove, in time, useful for risk assessment applications with this broad group of important contaminants with common receptor-mediated mechanisms of toxicity. These dosimetry models will have to be combined with emerging quantitative descriptions of cell and tissue responses to develop a complete biologically motivated risk assessment model. Some of the challenges of this approach have been outlined in this paper.

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APPENDIX

There are two mass balance differential equations for each tissue, one for tissue blood (tb) and another for tissue (t). Respectively,

$$dA_{tb}/dt = Q_t(C_a - C_{vt}) + PA_t(C_t/P_t - C_{vt}) \quad (1)$$

$$dA_t/dt = PA_t(C_{vt} - C_t/P_t) \quad (2)$$

For fat, slowly perfused, and richly perfused tissues, these equations are integrated for amount and tissue concentrations calculated by dividing amount (A_t) by volume (V_t).

$$A_t = \int_0^t dA_t \quad (3)$$

$$C_t = A_t/V_t \quad (4)$$

The tissue free (diffusible) concentration is calculated by dividing the tissue concentration (C_t) by the tissue partition coefficient (P_t)—Eq. (1) and (2). The liver equation [Eq. (5)] also contains a term for loss due to metabolism:

$$dA_l/dt = PA_l(C_{vl} - C_{lf}) - V_l kf(1 + \text{fold}(Cl_f/(C_{lf} + Kb_1))Cl_f \quad (5)$$

In the development of the mathematical description used here, there is the possibility for inducing metabolism in direct proportion to the fractional occupancy of the *Ah* receptor by dioxin. The extent of maximal induction as increase over the basal rate is controlled by the fold term in the above equation. For rat, this value was set to 1.0; it may vary in other species, such as the mouse.[13]

The total mass in the liver is then apportioned between free dioxin (C_{lf}) and bound forms of dioxin.

$$A_l = P_l V_l C_{lf} + [BM_1 C_{lf}/(KB_1 + C_{lf})] + [BM_2 C_{lf}/(KB_2 + C_{lf})] \quad (6)$$

The second term on the right of the equality is the concentration of the *Ah*-TCDD receptor complex (*Ah*-TCDD) and the third term is the concentration of the complex of TCDD and the induced binding protein.

The activity of P4501A1 at time, t, is calculated from the basal synthesis rate (K_0), the maximum increase in synthesis rate (K_{0max}), the *Ah* receptor-TCDD complex concentration, the complex-DNA dissociation constant (Kd_1), the appropriate Hill term (n_1), and the P4501A1 degradation rate constant (k_1).

$$d(\text{P4501A1}_t)/dt = K_0(1 + K_{0max}(Ah\text{-TCDD})^{n1}/((Ah\text{-TCDD})^{n1} + Kd_1^{n1})) - k_1\text{P4501A1}_t \quad (7)$$

The concentration of P4501A2 at any time, $BM2_t$, was calculated assuming an instantaneous adjustment of binding protein concentrations and *Ah*-TCDD levels,

$$BM2_t = BM2_0 + BM2_1(Ah\text{-TCDD})^n/((Ah\text{-TCDD})^n + Kd^n)) \quad (8)$$

where $BM2_1$ is the maximum induction of the binding protein. This formulation assumes very rapid induction of the binding proteins in these studies.

For the longer duration studies, the changes in total body weight and proportion of weight as fat compartment volume were included *via* table functions available in the ACSL software package.

For the single-dose studies in female rats,

	Simulated time (hr)		
	0	840	1344
Body weight (kg)	0.215	0.3	0.34
Fat (%bw)	0.07	0.09	0.13

For the repeated dose studies in male rats,

	Simulated time (hr)		
	0	840	1344
Body weight (kg)	0.35	0.39	0.41
Fat (%bw)	0.10	0.12	0.13

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APPENDIX C
PARAMETERS FOR ANALYZING PRENEOPLASTIC LESIONS AND TUMOR INCIDENCE IN RAT HEPATOCYTES

Under the assumptions of 8.2, when fitting the two-stage model to the Kociba et al. (1976) data, there is the potential to estimate as many as 24 parameters (four dose groups, each with its own two-stage model having six parameters). As discussed in this Appendix, the data given in this study make it impractical to estimate this many parameters with any degree of accuracy (Kopp-Schneider and Portier, 1991). To reduce the number of parameters in the model, β_N and δ_N were assumed to be known without error. Assuming that, on average and over finite time, the population of normal cells is effectively constant, it is reasonable to assume that $\beta_N = \delta_N$. This assumption may not hold true for TCDD because the labeling index for normal cells seems to increase with increasing exposure to TCDD (Lucier et al., 1991). However, to illustrate the use of the two-stage model, this assumption will be employed. Labeling data suggest that normal hepatocytes undergo mitosis at an average rate of one mitotic event per 300 days (a 2% labeling index for a 6-day labeling experiment), suggesting that $\beta_N = 3.333 \times 10^{-3}$. This value was used in the analysis that follows. Other values for $\beta_N = \delta_N$ were tried and had no effect on the resulting model parameters as long as it was assumed that changes in β_N do not imply changes in $\mu_{N\text{-}I}$ (Portier and Kopp-Schneider, 1991). The number of normal hepatocytes was assumed to be 6×10^8 for the female rat.

There are 16 parameters to be estimated from the tumor incidence data. Table C-1 shows one set of parameter estimates resulting from this fitting exercise (labeled "Unrestricted" under the model heading). Multiple attempts at fitting this model to the tumor incidence data indicated that the model was not identifiable; this means that there are an infinite number of parameter estimates that give effectively the same answer for these data. The main problem seems to be estimating $\mu_{N\text{-}I}$ and $\mu_{I\text{-}M}$ simultaneously (for example, in the high-dose group, any model with $\mu_{N\text{-}I} \cdot \mu_{I\text{-}M}$ equal to $3.4 \cdot 10^{-15}$ seemed to yield equivalent

Table C-1. Fitting the Two-Stage Model to the Tumor Incidence Data of Kociba et al. (1978) Using the Pathology of the Pathology Working Group (1990)

Model	Dose (ng/kg/day)	Parameters				Likelihood
		$\mu_{N\text{-}I}$	β_I	δ_I	$\mu_{I\text{-}M}$	
1. Unrestricted	0	1.14×10^{-10}	6.90×10^{-2}	3.14×10^{-2}	1.20×10^{-12}	
	1	9.48×10^{-10}	4.75×10^{-2}	1.58×10^{-2}	9/2-x1-$^{-15}$	
	10	9.85×10^{-10}	5.27×10^{-2}	2.44×10^{-2}	1.12×10^{-12}	
	100	3.33×10^{-5}	2.01×10^{-2}	1.61×10^{-2}	1.03×10^{-10}	-58.61
2. Unrestricted $\mu_{N\text{-}I}$	0	2.55×10^{-8}	1.75×10^{-2}	1.06×10^{-2}	1.31×10^{-9}	
	1	1.37×10^{-8}	—	—	—	
	10	1.36×10^{-7}	—	—	—	
	100	5.80×10^{-7}	—	—	—	-64.96
3. Unrestricted $\mu_{I\text{-}M}$	0	$1/31\times10^{-5}$	2.86×10^{-2}	2.16×10^{-2}	2.46×10^{-12}	
	1	—	—	—	1.37×10^{-12}	
	10	—	—	—	1.53×10^{-11}	
	100	—	—	—	5.81×10^{-11}	64.96
4. Unrestricted β_I	0	2.56×10^{-7}	1.24×10^{-2}	1.79×10^{-2}	3.02×10^{-9}	
	1	—	5.82×10^{-3}	—		
	10	—	2.15×10^{-2}	—		
	100	—	2.46×10^{-2}	—		-67.57
5. Unrestricted β_I (β_I in low-dose group set to zero)	0	2.56×10^{-7}	6.70×10^{-3}	1.23×10^{-2}	3.02×10^{-9}	
	1	—	0.0	—	—	
	10	—	1.59×10^{-2}	—	—	
	100	—	1.90×10^{-2}	—	—	67.57

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fits). The main reason for including the "Unrestricted" model in Table C-1 is that this model represents the best fit we can possibly achieve, and the likelihood (column 6) represents a best possible measure of goodness-of-fit. We will compare the likelihood for other models with this target likelihood.

The first restricted model to be considered is a model in which the effect of TCDD is only on the mutation rate from normal cells to initiated cells in the two-stage model. This model is fit by forcing β_I, δ_I, and $\mu_{I\text{-}M}$ to be constant across all dose groups and allowing $\mu_{N\text{-}I}$ to vary freely as dose changes. The parameter estimates for this model are given in Table C-1 as model (2). There are nine fewer parameters in this model than in the unrestricted model. Comparing the two likelihoods, it is evident that this model fits the data significantly worse than does the unrestricted model ($X^2_9 = 12.71$, $p<0.01$, as a technical note, since there is a problem with identifiability, the degrees-of-freedom, 9, for this X^2 random variable is inflated, which would inflate the p-value and the significance of the result would remain).

The problem with identifiability does not abate by restricting the parameters in the model. This is illustrated by the next model (3) in Table C-1. In this model, it is assumed the effect of TCDD is restricted to the rate of mutation from the initiated state to the malignant state. In this model, β_I, δ_I, and $\mu_{N\text{-}I}$ are held constant over all groups and $\mu_{I\text{-}M}$ changes as dose changes. Three points indicate the problem of nonidentifiability with this model. The first is that the likelihoods for this model (3) and the model changing $\mu_{N\text{-}I}$ (2) are identical. This indicates that the two models explain the same amount of noise in the data. The second point is that, even though the magnitude of the birth rate β_I has changed, the difference β_I - δ_I has not ($\sim 0.7\text{x}10^{-2}$). Further modeling with these data indicates this to be true over a wide range of β_I values. The third point is that, for each dose group, $\mu_{N\text{-}I} \cdot \mu_{I\text{-}M}$ is the same in the two models. Again, use of different fixed values for $\mu_{N\text{-}I}$ in model (3) and $\mu_{I\text{-}M}$ in model (2) supports this result.

The net result is that, unlike the results seen by Moolgavkar and Luebeck (1992) when fitting colon cancer data to the two-stage model, the magnitude of the mutation rate $\mu_{N\text{-}I}$ relative to $\mu_{I\text{-}M}$ cannot be addressed for the TCDD data. However, the relative change

in the product of these two mutation rates as a function of dose can be studied. This will be done later in this document.

A second restricted model is to consider that dioxin's only effect is on the birth rate of initiated cells (β_I). This is in fact the modeling approach used by Thorslund (1987). This model assumes that $\mu_{N\text{-}I}$, δ_I, and $\mu_{I\text{-}M}$ do not change as a function of exposure to dioxin. This model is given as model (4) in Table C-1. This model is also inconsistent with the data (likelihood = 67.57) and provides a fit that is worse than the mutational effect model given by (2). [Technical note: It is difficult to directly compare these two models since they constitute nonnested models.] This implies that the effect of TCDD on tumor incidence in these rodents is more likely due to a mutational effect than a mitogenic effect on initiated cells. Caution must be taken when interpreting this result. First, no statistical confidence can be placed on this statement, so the observed difference in the two models may be due solely to random change. Second, this statement is only justified within the restricted context of this two-stage model of carcinogenesis. If any assumptions of the model are incorrect (independent cell action, constant rates, two stages, etc.), the interpretation based on this model could be biased.

The problem with identifiable parameters also remains with this formulation of the model. This is illustrated by the fifth model in Table C-1. In this model, the birth rate in the lowest dose group was fixed to be zero. The resulting likelihood and estimates of $\mu_{N\text{-}I}$ and $\mu_{I\text{-}M}$ remained the same. The estimated birth rates (β_I and death rate δ_I) changed, but the difference between the two in the various dose groups did not change. Repeated applications of this formulation of the model confirmed this problem of identifiability in this case.

With this problem of identifiability, there are basically only two parameters to be estimated for the two-stage model for each dose group from these data. These are the mutation parameter, given by $\mu = \mu_{N\text{-}I} \cdot \mu_{I\text{-}M}$, and the proliferation parameter, given by $\Pi = \beta_I - \delta_I$. Table C-2 gives the estimates of these parameters for the simple models considered here. The first model corresponds to the case where we allow both the mutation parameter and the proliferation parameter to change as a function of dose (model (1) in Table

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Table C-2. Net Parameter Estimates From the Two-Stage Model of Carcinogenesis

Two-stage parameters changed	Doses				Other parameters
	Control	1 ng/kg/day	10 ng/kg/day	100 ng/kg/day	
Both	1.37×10^{-22} 0.0376	0.077×10^{-22} 0.0317	8.86×10^{-22} 0.0283	3.43×10^{-15} 0.0040	
Both (proliferation rate <0.02)	2.80×10^{-20} 0.02	1.91×10^{-20} 0.0196	18.79×10^{-20} 0.0196	3.58×10^{-15} 0.00198	
Mutation rates	3.34×10^{-17}	1.79×10^{-17}	17.8×10^{-17}	76.0×10^{-17}	Proliferation rate = 6.90×10^{-3}
Proliferation rates	-0.0055	-0.0121	0.00362	0.00669	Mutation rate = 7.73×10^{-16}

C-1). For this model, as dose increases, the mutation parameter drops to one-half the control value for the 1 ng/kg/day dose group and increases substantially in the remaining two dose groups (6.5-fold for 10 ng/kg/day and 25×10^6-fold for 100 ng/kg/day). The hepatocyte replication parameter drops as a function of dose. In the 100 ng/kg/day dose group, the replication parameter is small relative to control (tenfold smaller) to adjust for the much larger mutation parameter. The reason for this particular pattern is clear if one studies the tumor incidence data from the Kociba et al. (1978) study. Over time, the tumor incidence in the highest dose group is larger than the others (the mutation parameter and the replication parameter combine to control the magnitude of the response) but climb less steeply with time (this is controlled by the replication parameter).

The replication rates in the control group and the two lowest dose groups are very high. Assuming the death rate is zero, this would correspond to a labeling index of 40.9% for a 7-day labeling experiment in the control animals. If the death rate is >0, the labeling index would be even larger. Also, if one cell entered the initiated state on day 1, by 730 days (2 years), one would expect a clone of size 8×10^{11}, ~ 140 times the size of a normal rat liver. To control for this problem, the same model was fit to the data with the replication parameter constrained to be less than 0.02 (a first-day clone would be expected to have a size of 2×10^6 by study end). This resulted in the same pattern of mutation parameters but with a mutation parameter two orders of magnitude larger in the control and two lowest dose groups. For all three groups, the replication parameter was estimated to be at the boundary (0.02). The parameters for the high-dose group did not change.

The model in which the replication parameter is constant across dose groups and the mutation parameter changes for each new dose is given in row 3 ("Mutation rates") of Table C-2. The pattern in this case is very clear and matches the observed cumulative tumor incidence well, with the mutation rate dropping in the 1 ng/kg/day dose group then rising in the remaining groups. This observed drop is not statistically significant from the control mutation parameter (likelihood ratio test, $p>0.05$). The constant replication rate (6.9×10^{-3}) is reasonable and is not likely to produce unrealistically large clones of initiated cells. As

mentioned before, this model provided a significantly worse fit to the data than did the unrestricted model.

Finally, the estimated parameters for the model in which the replication parameter changed with dose and the mutation parameter was constant over all doses are given by the last row ("Proliferation Rates") in Table C-2. The change in replication rates mirrors the tumor response in the same manner as was seen for the mutation rates. The replication rates are of reasonable size and should not produce impossibly large clones. As mentioned earlier, this model does not fit the data as well as the unrestricted model or as well as the model with fixed replication rate over dose and varying mutation rate over dose.

There is other evidence that can be used to examine the adequacy of this model for tumor incidence from exposure to TCDD. Lucier and colleagues recently conducted an initiation/promotion study in female Sprague-Dawley rats (Tritscher et al., 1992; Sewall et al., 1993; Maronpot et al., 1993). In this study, they measured number and size of preneoplastic foci in liver sections. It has been suggested that the cells in these lesions correspond to the initiated cells in the two-stage model of carcinogenesis. If this is true, it is possible to apply the methods of Dewanji et al. (1989) to these data to analyze the growth characteristics of these cells (Moolgavkar et al., 1990). However, as before, we run into the problem of nonidentifiability. Since the data in these studies were collected at only one time point, it may not be possible to estimate all of the parameters in the first half of the two-stage model (i.e., $\mu_{N\text{-}I}$, β_I, and δ_I) and get a unique solution. For each dose group, one of these parameters should come from outside information or continuous dose-response models should be assumed.

Maronpot et al. (1993) measured the rate of cell proliferation in these rodents (Tritscher et al., 1992) using immunocytochemical detection of cells that had undergone replicative DNA synthesis. The average labeling index by dose group is given in Table C-3 for the uninitiated animals (saline controls) in this study. It is seen there is a slight drop in mean labeling index ($p > 0.05$) from control to the group given 3.5 ng/kg/day. The labeling index then increases with increasing dose. Under the assumption of a linear birth death

Table C-3. Results From Two-Stage Model for Hepatocarcinogenesis in Rats

Model	Parameter	TCDD Dose Administered				
		Control	3.5 ng/kg/day	10.7 ng/kg/day	35.7 ng/kg/day	125 ng/kg/day
Labeling index data and calculations	Labeling index	3.41	3.22	4.87	5.31	7.09
	Birth rates	2.75×10^{-3}	2.34×10^{-3}	3.56×10^{-3}	3.90×10^{-3}	5.26×10^{-3}
	Relative change (%)	0	-14.9	29.5	41.8	91.3
	Scaled birth rates	2.06×10^{-2}	1.76×10^{-2}	2.67×10^{-2}	2.93×10^{-2}	3.95×10^{-2}

Source: Lucier et al., 1991; Tritscher et al., 1992; Maronpot et al., 1993.

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process, it is possible to convert these labeling indices into estimated birth rates for these cells using the formula:

$$\beta = \frac{1}{2t} \ell_n \left[\frac{1}{1 - LI} \right]$$

where β is the birth rate, t is the number of days over which labeling was done, and LI is the labeling index (Moolgavkar and Luebeck, 1992). This conversion is given in row 2 of Table C-3. These rates are in nonfocal hepatocytes (normal cells) and correspond to an average of one to two births per year per hepatocyte. TCDD seems to double the control birth rate for a dose of 125 ng/kg/day (a relative change of almost 100%--row 3 of Table C-3).

Maronpot et al. (1993) also calculated the labeling index in focal hepatocytes in the high-dose group from this study. The mean LI for animals in the initiated group exposed to 125 ng/kg/day of TCDD was 42.5%, which corresponds to a birth rate of 0.0395. The ratio of birth rate in nonfocal cells to birth rate in focal cells is 0.0395/0.00526 = 7.5. Assuming this ratio is constant over all dose groups, we can rescale the birth rates in the remaining dose groups to correspond to birth rates for focal cells resulting in the rates shown as row 4 in Table C-3.

Using the same method as that used by Moolgavkar et al. (1990), given these birth rates, it is possible to estimate $\mu_{N\text{-}I}$ and δ_I for the focal lesion data of Lucier et al. (1991). The resulting model did not fit these focal lesion data.

To get a handle on how large the labeling index would have to be to result in a reasonably good fit to the data, the model was again fit to the liver foci data, allowing β_I to roam freely and the ratio δ_I/β_I to be <0. The resulting parameter estimates are given in Table C-4.

Table C-4. Estimated Model Parameters in the Two-Stage Mathematical Model of Carcinogenesis (Figure 1) for the Noninitiated (Saline) Animal

Dose[a]	$X_0\mu_1$[b] (mutation rate)	β[c] (birth rate)	ρ[d] (death/birth)	Log-likelihood
A. Fully nonparametric in dose				
Control (0.0)	$3.851\ 10^{-4}$	$3.041\ 10^{-2}$	$6.077\ 10^{-2}$	282.79
3.5	$4.346\ 10^{-4}$	$3.548\ 10^{-2}$	$6.062\ 10^{-2}$	
10.7	$3.860\ 10^{-4}$	$3.749\ 10^{-2}$	$1.267\ 10^{-1}$	
35.7	$9.964\ 10^{-4}$	$3.576\ 10^{-2}$	$3.354\ 10^{-2}$	
125.0	$7.0435\ 10^{-4}$	$3.664\ 10^{-2}$	$6.210\ 10^{-2}$	
B. Constant mutation rate, other parameters nonparametric in dose (pure promoter)				
Control (0.0)	$8.423\ 10^{-4}$	$6.778\ 10^{-2}$	$6.385\ 10^{-1}$	281.62 (p=0.673)
3.5		$6.045\ 10^{-2}$	$4.998\ 10^{-1}$	
10.7		$9.510\ 10^{-2}$	$6.988\ 10^{-1}$	
35.7		$3.509\ 10^{-2}$	$0.000\ 10^{-1}$	
125.0		$3.372\ 10^{-2}$	$0.000\ 10^{-1}$	
C. Constant ratio (ρ) and birth rate (β), nonparametric for $X_0\mu_1$ (pure initiator)				
Control (0.0)	$0.241\ 10^{-3}$	$3.578\ 10^{-2}$	$5.386\ 10^{-2}$	280.78 (p=0.855)
3.5	$0.415\ 10^{-3}$			
10.7	$0.339\ 10^{-3}$			
35.7	$1.066\ 10^{-3}$			
125.0	$0.731\ 10^{-3}$			
D. Constant ratio (ρ) and hyperbolic[e] birth rate (β), $X_0\mu_1$				
Intercept	$3.070\ 10^{-4}$	$3.003\ 10^{-2}$	$3.825\ 10^{-3}$	276.73 (p=0.146)
V_{max}	$5.909\ 10^{-4}$	$4.948\ 10^{-3}$		
k_d	18.17	2.20		

[a]ng/kg body weight/day.
[b]Mutations per day per mm^3 of liver.
[c]Births per cell per day.
[d]Deaths per birth.
[e]Parametric form is rate (dose) = intercept + E_{max} dose/($D_{0.5}$+dose).

There is considerable instability in the estimated model parameters for the nonparametric model (A) in Table C-4, possibly due to the fact that there may not be sufficient data in each dose group to allow for the estimation of individual parameters. This is especially true for the low-dose groups in which there are few focal lesions and they are generally of small size. However, the model does fit the data rather well.

The first task is to test if the effect of dose, within the context of this model, is significant or not. This can be restated as the hypothesis that one set of model parameters will fit all five treatment groups. This is accomplished by fitting the same two-stage model to all five data sets, treating them as one large experimental group (this model is not shown in the table). Using a likelihood ratio test, there is a significant change in the two-stage model parameters with $p<0.001$. The next step is to attempt to simplify the final model to include fewer parameters and provide a continuous description of the effect of dose on the various model parameters.

Based on the existing understanding of how TCDD induces PGST+ foci in this biological two-stage model, it would be natural to assume that $X1_0\mu_1$, the mutation rate from normal cells to initiated cells, does not change as a function of dose. Model B in Table C-4 addresses this basic model. In fitting a new model to these data, the first task is to assess, using a proper statistical test, if this model fits the data as well as the original model. Using the likelihoods, it is possible to test this hypothesis using a likelihood ratio test. For these data, this yields $p=0.673$, indicating that a model with a constant mutation rate fits the data as well as the fully nonparametric model and suggesting that TCDD at most causes a marginal increase in either X_0, μ_1, or both. Maronpot et al. (1992) noted some evidence of hepatotoxicity via histopathological changes, small increases in relative liver weight and alterations in serum chemistry. The increases in relative liver weight would suggest the effect may be on X_0, but the hepatocytes were also enlarged, making this determination difficult. As discussed in Kohn et al. (1993) and Portier et al. (1993), it is possible for TCDD to have a small effect on mutations in these animals via a secondary pathway. This finding is consistent with that conjecture.

Over the entire dose range, the ratio of death rate to birth rate follows an erratic pattern that varies over several orders of magnitude. Sensitivity analysis shows that the likelihood is insensitive to rather large changes in the ratio, suggesting it may be possible to get an adequate fit to these data with a constant value for ρ across all dose groups. Analyses done by Moolgavkar and Luebeck (1992) and Luebeck et al. (1991) relied on this assumption. A model with ρ constant across all dose groups indicates that there is no effect of TCDD on the ratio of the death rate to the birth rate of PGST+ foci in uninitiated animals.

Model C, in contrast to model B, tests the hypothesis that there is no effect of TCDD on growth rates of initiated cells. The results are not significant, indicating a pure initiation model fits these data as well as a pure promotion model. Model C also provides a much clearer pattern to the effect of dose on the mutation rate and birth rate for the two-stage model. The mutation rate ($X_0\mu_1$) basically increases until the highest dose group where there is a slight decrease. This decrease may be due to a single animal in the 35.7 ng/kg/day dose group. This would suggest that we might model the mutation rate (and the birth rate) by the hyperbolic function; for the mutation rate, this would be given as

$$X_0\mu_1(\text{dose}) = \text{intercept} + V_{max}\,\text{dose}/(k_d + \text{dose}) \qquad [1]$$

A similar model is used for the birth rate. The parameter estimates for this model are presented in Table C-4 as model D. A likelihood ratio test indicates that this model provides an adequate fit to these data ($p=0.146$).

Several other models were fit to these data. These models were various combinations of constant, nonparametric, linear, and hyperbolic dose-response functions for the three rate functions ($X_0\mu_1$, ß, and ρ) for the two-stage model. The model given in Table C-4 as model D seems to be the best parameterization that agrees with the data and the biological understanding of the carcinogenicity of TCDD.

The DEN-initiated animals must be handled differently from the noninitiated group. It is believed that initiation with DEN results in a number of the normal cells undergoing mutation. This process is assumed to occur in a very short period. To accommodate initiation, Luebeck et al. (1991) introduced a second mutation rate process at the start of the experiment. These data were fit using the methods of Luebeck et al (1991). The resulting model agrees very closely with the model for the saline-treated animals, the main difference being a larger mutation rate.

The calculations have hidden assumptions that could have a bearing on the interpretation of the results. First, we have ignored the changes in labeling index in the normal hepatocytes. This could change the size of the pool of normal hepatocytes, which is assumed constant for these calculations. This might alter the parameter estimates. In addition, these changes in the rate of replication in nonfocal tissue could alter the mutation rate $\mu_{N\text{-}I}$ (Portier and Kopp-Schneider, 1991). As for tumor incidence, the model assumes constant rates and independent cell kinetics. Finally, the large estimated percentage of cells that are initiated can only imply most of these initiated cells are single cells and cannot be observed on histopathological examination. This has not been verified. The analysis is very sensitive to the choice of values for the radius of a cell (Moolgavkar et al., 1990) and to the minimum size of a detectable focus. These issues cannot be discussed fully in this brief document.

APPENDIX D
CONSIDERATIONS FOR USING DOSE SURROGATES IN ESTIMATING TUMOR INCIDENCE

It is possible to compare the parameters estimated for the two-stage model to predictions from the PB-PK models to try to locate a reasonable mechanistic link between the two classes of models, to aid in species extrapolation, and to help guide us in choosing the most appropriate curvature for low-dose extrapolation. The two-stage model parameters that were estimated from the tumor incidence data are compared to 14 dose surrogates from the three main PB-PK models reviewed earlier. Leung et al. (1990a) suggested using occupancy of the cytosolic (Ah) receptor, averaged over the length of the study. They also suggested using average binding to the microsomal proteins. These predictions are shown in Table D-1 for the study of Kociba et al. (1978), along with the predicted final concentration of TCDD in the liver of these animals. The correlation coefficients of these parameters with the two-stage model parameters are shown in the last three columns of Table D-1 under "Both," "Mut. Rates," and "Prol. Rates." It is clear that these three surrogates correlate well with all but the two-stage model in which the effects of TCDD were treated as pure promotional effects. The high correlations and low p-values for these correlations are driven by the order of magnitude differences in the parameters and the dose surrogates. (Caution should be used in judging these correlations to carry any weight of scientific evidence.)

Andersen et al. (1993b) suggested four dose surrogates to be used in any risk assessment for TCDD. These are based on free TCDD in the liver, total TCDD in the liver, amount of induced CYPIA1, and amount of induced CYPIA2. As for Leung et al. (1990a), these are integrated over the lifespan of the animal (in this case, total integrated rather than average, but this will not affect the correlations). As for Leung et al. (1990a), the correlation with all two-stage model formulations/parameters is significantly different from zero for three of the surrogate doses (excluding CYPIA2) and all but the promotion-only

Table D-1. A Comparison of Dose Surrogates With Parameters Estimated From the Two-Stage Model of Carcinogenesis

Dose surrogate	Dose				Correlation[a]		
	Control	1 ng/kg/day	10 ng/kg/day	100 ng/kg/day	Both	Mut. Rates	Prol. Rates
Leung et al. (1990a)							
Concentration	0.0	0.3	4.4	69.4	1.00^{++} -1.00^{++}	0.99^{+}	0.70
Ah-receptor occupancy	0	2	17	61	0.96^{+} -0.96^{+}	1.00^{++}	0.81
Microsomal binding	25	29	132	132	0.96^{+} -0.96^{+}	1.00^{++}	0.81
Andersen et al. (1993b)							
Free TCDD in liver	0	6.4	53	420	0.99^{++} -0.99^{++}	1.00^{++}	0.73
Total TCDD in liver	0	590	11,000	120,000	1.00^{++} -1.00^{++}	1.00^{++}	0.72
Induced CYPIA1	0	3,900	270,000	200,000	0.99^{++} -0.99^{+}	1.00^{++}	0.74
Induced CYPIA2	0	57,000	210,000	320,000	0.79 -0.79	0.89	0.87
Kohn et al. (1993)							
Free TCDD in liver	0	0.1237	0.7663	5.0522	0.99^{+} -0.99^{+}	1.00^{++}	0.74
Ah-receptor/TCDD complex	0	1.0202	8.8617	49.352	0.99^{+} -0.98^{+}	1.00^{++}	0.76
CYPIA2/TCDD complex	0	1.6857	27.3142	464.85	1.00^{++} -1.00^{++}	0.99^{+}	0.69
TGF-α	0	0.005899	0.0540 8	0.3788	0.99^{++} -0.98^{+}	1.00^{++}	0.74
Internalized EGF receptor	0	0.4829	3.7213	13.668	0.97^{+} -0.96	1.00^{++}	0.83
Total CYPIA1	190	2,198	14,213	42,196	0.95 -0.95	0.99^{++}	0.83
Total CYPIA2	4,517	5,999	15,747	44,579	0.96^{+} -0.96^{+}	1.00^{++}	0.81

[a]Pearson correlation coefficient -+ indicates statistical significance at the 0.05 level, ++ indicates significance at the 0.01 level (caution should be used in applying the p values too literally due to the small sample sizes involved).

TCDD model. The correlation of induced CYPIA2 with the two-stage model parameters is not significantly different from zero for all two-stage models. Note that the highly nonlinear CYPIA1 curve correlates well with the first two two-stage models. If this were used as a dose surrogate for TCDD toxicity (in the independent framework chosen by Andersen et al. [1993b]), the resulting low-dose risks would be substantially smaller than those of any other dose surrogate. Induced CYPIA2 fails to correlate due to the strong saturation seen at the two highest doses; an effect not observed in the two-stage model fits.

Kohn et al. (1993; Appendix A) did not suggest specific dose surrogates but did suggest mechanisms that naturally lead to the choice of certain surrogates. Seven such surrogates are given in Table D-1: free liver TCDD, Ah-receptor bound TCDD, CYPIA2-bound TCDD, integrated TGF-α, internalized EGF receptor, integrated CYPIA1, and integrated CYPIA2. With the exception of integrated CYPIA1, all dose surrogates correlated as well with the two-stage parameters as the dose surrogates from the other two PB-PK models. None of the surrogates resulted in correlations with the promotion model, which were significantly >0. The internalized EGF receptor correlates well with the promotion model but not significantly better than 0 and not better than induced CYPIA2 in the Andersen et al. (1993b; Appendix B) model. Total CYPIA2, which Kohn et al. (1993) suggest could be tied to secondary mutagenic effects of TCDD, correlates well with the mutation model. All of the dose surrogates predicted by the Kohn et al. (1993) model have positive slope at dose 0 and would behave, in the low-dose region, as a linear model. Risk estimates utilizing these dose surrogates and the two-stage model would likely result in a less than tenfold change in risk over what would be estimated by applying a one-stage model to the Kociba et al. (1978) data.

It should be possible to correlate these same dose surrogates with the model parameters arising from the liver foci analysis. However, none of the authors calculated dose surrogates for this design situation. This can be done at a later time.

One final point on dose surrogates: The choice of which measure of exposure one decides to correlate with which measure of effect is a somewhat arbitrary decision. The choices above were mostly chosen because they have traditionally been used in this context. However, there is no pressing mechanistic reason why these particular choices are best. The major two variables in this decision are which end points(s) and how to consider time factors. For example, we could also have considered induced amount of Ah receptor as a dose surrogate; there is no a priori reason to exclude it. It is also possible to integrate over shorter periods of time. This is not really mechanistically justifiable for TCDD, but for other compounds, which show a short mitogenic effect or get rapidly metabolized into toxic compounds of brief duration, shorter integration periods would be more appropriate. Thus, some thought should be given to a choice of dose surrogate based on mechanistic considerations. The paper by Kohn et al. (1993) discussing their mechanistic model can be used to provide considerable direction on this topic (see Appendix A).

APPENDIX E

TETRACHLORODIBENZO-P-DIOXIN EMPIRICAL RELATIONSHIPS FOR NON-CANCER ENDPOINTS

Lynne F. McGrath, K.R.Cooper, P.Georgopoulos, and M.A. Gallo
Environmental and Occupational Health Sciences Institute
UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ

I. INTRODUCTION

Dose-response assessment is an essential element in determining health risks associated with environmental contaminants. The evaluation of dose-response requires information on a range of quantifiable doses or exposures and corresponding measured responses. The study of adverse effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the use of dioxins as tools to elucidate underlying biochemical and physiological mechanisms of cellular action are pivotal in the overall evaluation of dioxin exposure. The determination of the shape, slope and of the dose-response functions of TCDD for several endpoints, over multiple time points and in multiple species, is critical to the understanding of the potential human health risks and exposure to dioxin.

Dose-response relationships for several endpoints can be established using human data (Poole, this document) however quantification of exposure can be difficult. The assessment of TCDD presented here primarily employs controlled animal studies in which administered dose is known and delivered dose is either measured or can be estimated. The endpoints described were those found to have some consistency across species and strains. This extensive review of dose-response relationships has been completed to better elucidate key biomarkers or surrogates for cancer and other life threatening diseases.

TCDD and members of this family of compounds are exogenous ligands for the Ah receptor(AhR). This receptor had originally been hypothesized to be a member of the nuclear hormone receptor family. The overall hypothesis of TCDD action, put forth by several groups, (see

Whitlock chapter this document) can be simplified as follows: TCDD and the other members of the family (ligands) enter the cell by mass action, bind to the AhR (receptor), the receptor-ligand complex translocates to the nucleus via specific transport proteins and subsequently binds to specific sequences on the gene. This in turn evokes the production (or perhaps the suppression) of several mRNA species. Recent results have identified a specific consensus sequence on the gene which has been named the xenobiotic response element (XRE)(Whitlock,1991, Denison, 1991). In addition to the DNA sequence identification, the structure and amino acid sequence of the AhR protein was reported by Burbach et al., (1992). Both the XRE(s) and the structure of the AhR are analogous to the steroid receptors and their respective genomic response elements. This similarity is important in regard to biological models of TCDD action and risk assessment.

Dose-response relationships have been established for several endpoints in intact and surgically altered animals. In vitro experiments have been used to determine critical concentration and structural relationships for TCDD effects at the cellular and molecular level. In the vast majority of these studies the role of the AhR-ligand interaction has been essential but not necessarily sufficient to evoke a detectable biological response.

Although all toxic endpoints for TCDD may not be Ah locus mediated the evaluation of dose response allows us to conclude that the critically sensitive events are Ah receptor mediated. The multiple steps in receptor mediated processes suggest that the dose-response relationships are not linear. This series of cellular events can be best modeled as a simple series of log-differential equations which basically take the form of Michaelis-Menten hypotheses, which include Michaelis-Menten and Hill functions. This format allows for slope estimates, as well as inhibition and saturation phenomena. Hence, if curvilinearity of the dose-response exists, it should be possible

to model using kinetic equations. Indeed, this may well be the case for some end effects of TCDD, however, recent rodent experiments suggest the induction of cytochrome P-450IA1 (CypIA1) is linear through zero (curve fitting with a Hill function) using long term exposure and very low doses (Lucier et al., 1991a).

Low dose phenomenon like thymic atrophy and immunotoxicity have also been described and are the result of multistep processes in which many of the steps are unknown, and others are non-specific. Non-cancer endpoints have generally not been subject to dose-response modeling such as that used for assessment of cancer endpoints although many have been consistently reported after TCDD administration. The focus of this dose-response assessment is on toxic endpoints for which a mechanism has been hypothesized and on the description of low dose effects. In addition, there is the practical criteria of using a satisfactory number of dose points.

To improve our understanding of non-cancer endpoints and to elucidate possible mechanistic relationships between endpoints, several simple functional forms were used in the empirical analysis of relationships between TCDD dose and non-cancer endpoints. Throughout the document datasets have been selected for a more rigorous dose-response assessment. The criteria used for inclusion is endpoint and dataset specific, but generally it depends on the number and range of dose points. Specific rational for selection is described with the specific endpoint. Because of the exploratory nature of this analysis the number of assumptions regarding dose-response relationships were kept to a minimum. Each functional form used will be briefly discussed with comments on the nature of these assumptions. For a more extensive mathematically detailed explanation of these functions see Appendix.

Initially a x-y plot of the data was made to provide a visual examination of dose-response.

Attempts to use the Linear Multistage Model for fitting the dose response did not give satisfactory correlation coefficients, in addition the underlying biological assumptions for this model would not be appropriately applied to the non-cancer endpoints described in this document. Although these graphs are not included in this document they were used as a first step in the analysis. The first and simplest model used to describe selected data sets was the linear function.

The Linear Model

$$y = mx + b$$

(x = dose; y = response; m = slope; b = Background response)

Many dose-response relationships in pharmacology and toxicology are known to fit the linear model. For example, the elimination of some chemicals (ie., chloroform) from plasma obeys 1st order kinetics and as such is modeled as a linear function. In many cases the actual dose-response relationships are nonlinear but appropriate transformations of the independent and dependent variables are used to obtain a linear relationship. For example, Scatchard and Lineweaver-Burke plots represents ligand-receptor binding using equations in a linear form however, the binding phenomenon is non-linear (Tallarida and Jacob, 1979). The success of the linear fit is typically evaluated via least squares criteria resulting in an R^2 value which quantifies the "goodness of fit". However, in the case of a nonlinear phenomenon that has been transformed into a linear relationship, the transformation introduces a bias in the R^2 estimate. (These R^2 values are not directly compared to those derived form linear functions).

The second functional form used to evaluate the TCDD dose-response relationships was the Michaelis-Menten equation. This is a hyperbolic function often used to fit the relationship between

enzyme-substrate or receptor ligand interactions.

The Michaelis-Menten Model

$$y = ax / (b+x)$$

(x = dose; y = response; a = maximum response; b = concentration of x at 1/2 maximum response)

The assumption inherent when plotting the ligand-receptor binding relationship is that there is a single rate function that represents the relationship between the free receptor and the bound receptor over specified doses, and that a finite number of binding sites must be occupied to initiate a response and that saturation of the receptors occurs, and that this maximum interaction is reached at some finite dose (Goldstein et al., 1974).

The third function used was the Power Law formalism. The relevant equation is nonlinear and includes a power (not necessarily an integer) as a term in the function.

Power Law Function

$$y = ax^g$$

(x = dose; y = response; g = kinetic order; a = limiting response)

This functional form has been historically used to represent a variety of nonlinear biological phenomenon including the saturation of, or Synergy in, enzyme systems; thus the name "S-Systems" (Savageau, 1991). This formalism has been used to describe processes obeying power laws which provides adequate descriptions of many nonlinear interactions that occur in nature. It is not a surrogate for Michaelis-Menten function, but provides an approximation of individual biological interactions or associations. The exponent in the Power Law function ("g") is related to the pharmacological kinetic order (rate of response) of the observed process. If the "g" value is

greater that 1 the response is superlinear and positive cooperativity is assumed, whereas is the response is less that 1 and sublinear, then negative cooperativity is assumed. The Power Law has fewer degrees of freedom than the Hill function (see below) and it can fit subsets of doses allowing for different reaction rates to be determined. Although it has not been specifically applied to toxicological results for TCDD, the generalized approach of the Power Law formalism is compatible with the pleiotropic responses seen after TCDD administration (Savageau, 1991). The Hill and Michaelis-Menten functions describe a response over an entire dose range. However, the Power Law, when applied to subsets of a data, may provide a mechanism for describing changes in underlying biology from low to high doses.

The fourth function used in this analysis was the Hill function. This nonlinear function is a generalization of the Michaelis-Menten equation as it incorporates in it the flexibility of the Power Law approach.

The Hill Function

$$y = (ax^g) / (b + x^g)$$

(x = dose;y = response;g = kinetic order;b = concentration of x at 1/2 maximum response;a = maximum response)

It has been used to model receptor-ligand interactions, and because of its wide acceptance and flexibility an attempt to fit this formalism was made for selected datasets (Boeynaems and Dumont, 1975). As in the Power Law formalism, the value of the "g" exponent in the Hill plot determines the behavior of dose response; if greater than 1 the response is superlinear, if the exponent is less

than 1 it is sublinear[1]. The Hill plot is not as simple as the Power Law function because of the greater number of variables. The Hill plot assumes saturation at the high doses, as well as an "inertial"[2] type of response at low doses. If the underlying biology of a system indicates that saturation occurs at high doses, then the Power Law function, because it is exponential, does not accurately describe the high dose phenomenon.

In the analysis of empirical relationships selected data sets were tested using various functional fits. The Michaelis-Menten and Hill functions were adopted for use because it is the most widely used model that assumed receptor-ligand or substrate-enzyme interactions. The nonlinear model used to fit all data sets was the Power Law function because of its simplicity. Initially these functions were used to fit the percent-response form of data which was used as a relative normalization procedure. Subsequently, these functions were used to fit raw data. The Power Law function, because it was not constrained by underlying assumptions, was then used to fit ranges of doses to look for changes in the exponent possibly detecting rate changes. Analysis of the relationships for specific endpoints are discussed in detail throughout this chapter with the descriptions of the specific endpoints.

Because many of the data sets have few values much of the available statistical analysis (mean square error of fit, analysis of co-linearity) were not performed. Hypothesis testing to evaluate curve fits was also limited by the number of data points. These data could be made

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[1] A superlinear dose-response could generally suggest positive cooperativity or synergy through the measured dose-response range, whereas, a sublinear dose response could imply negative cooperativity or an inhibitory effect.

[2] The definition of inertia is " a property of matter by which it remains at rest or in some uniform motion in the same direction unless acted upon by a net external force" Merkin, 1985

more robust by the analysis of raw data. Since rigorous statistical analysis was not applied to the data three criteria were used to conclude a "poor fit" existed. First a low R^2 or a high chi square value, second, visual observation of the curve fit, and third the inability to fit the data to a particular function.

Table I: Summary of Dose Response Functions

LINEAR MODEL	Assumes biological response is linear.	$R^2 > 0.8$ typically assumes good fit
MICHAELIS-MENTEN FUNCTION	1. Assumes a non-linear biological phenomenon. 2. Assumes minimal or no response at low doses. 3. Assumes maximum activity is reached in dose range.	Chi Square "goodness of fit" dependent on degrees of freedom
HILL FUNCTION	1. Assumes a non-linear biological phenomenon. 2. Assumes low dose response is negligible or "inertial". 3. Assumes saturation or maximal activity is reached at high doses.	Chi Square "goodness of fit" dependent on degrees of freedom
POWER LAW FUNCTION	1. Assumes a non-linear biological phenomenon. 2. Assumes synergy or cooperativity.	$R^2 > 0.8$ typically suggests a good fit; however it should be taken into account that the R^2 estimate is logarithmically biased.

In assessing potential health risks, an analysis of dose-response provides an estimate of the severity of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. The focus of this dose-response assessment is on studies using multiple doses and on evaluating multiple endpoints in several species. The hope, in evaluating responses in a variety of species, is that consistency will add confidence to the estimates of human health risks subsequently made. This report emphasizes reevaluation of effects that occur at low doses; these being the most relevant to environmental health. Responses at higher doses are also included to demonstrate species differences and estimations of what might occur under incidental high exposures (such as industrial accidents). Experimental evidence indicates that TCDD is extremely toxic in some systems at low doses. However, although qualitatively similar, the magnitude of response is species and strain specific.

II. ACUTE TOXICITY

A large body of data exists describing the acute toxicity of TCDD in a variety of species. Acute data on lethality is briefly described here and will be referred to throughout the document. Acute responses are generally considered Ah receptor mediated segregating with the Ah locus. In this analysis of non-cancer endpoints this acute toxicity information is used as a basis of comparing tissue, species and strain sensitivity. Sensitivities that differ from those listed in Table II will be probed for endpoint specific factors that may modify the response.

The acute responses listed in Table II are LD_{50}'s which vary almost 10,000 fold. The accumulated levels of TCDD causing lethality after continuous administration are consistent with the levels causing effects after single exposure which strongly suggests that over short periods of time the total body burden is the rate limiting factor in morbidity. It is generally recognized (Poland and Knutson, 1982) that the guinea pig is most sensitive to the lethal effects of TCDD followed by the rat (sensitive strain), the mouse (sensitive strains), rat and mice (non-sensitive strains) and by the hamster. However, the rhesus monkey (see below) may be more sensitive than the guinea pig. Because of the variety of protocols used to assess acute toxicity, several studies are reviewed here. In a continuous feeding study, rhesus monkeys were administered 500 ppt TCDD for 9 months (Allen et. al, 1977). Four months after this treatment period 4 of the 8 animals died after receiving an estimated total dose of 18.6 ug/animal (approximately 3.5 ug TCDD/kg or 0.013 ug TCDD/kg/day). Based on these data the LD_{50} may be considerably less than the 70 ug TCDD/kg (lowest dose tested) reported by McConnell et al., (1978) (Table II). In a continuous feeding study in Hartley guinea pigs, 5 of 10 males died after a total dose administration of 0.6 ug TCDD/kg to 1.17 ug TCDD/kg. Four of 10 females died after a total dose administration

Table II
Comparative Acute Toxicity of TCDD
LD50 Values

Species	Sex		LD50 ug TCDD/kg	Time to Death	Reference
Hartley guinea pig	Male	oral	0.6	5-34 days	Schwetz,1973
Hartley guinea pig	female	oral	2.5	42 days	Silkworth et al., 1982
Hartley guinea pig	male	oral	2.1	19-42 days	Schwetz et al., 1973
Golden syrian Hamster	male	i.p.	>3000	50 days	Olson, et al., 1980
Golden syrian Hamster		oral	1157	50 days	Olson et al., 1980
Golden syrian Hamster	male	oral	5051	26-43 days	Henck et al., 1981
Sherman (Spartan) rat	male	oral	22	9-27 days	Schwetz et al., 1973
Sherman (Spartan) rat	female	oral	45	13-43 days	Schwetz et al., 1973
Han/Wistar rat Long Evans	male	i.p.	>3000 10		Pohjanvirta et al., 1990
Fischer rat Charles River Fischer rat Harlan Fischer rat Fredrick Cancer Center CD rat Charles River	male	oral	164 340 303 297	24.8 ± 0.6 28.3 ± 0.5 25.9 ± 0.8 24.5 ± 1.0	Walden, 1985
New Zealand albino rabbit	male and female	oral	115	6-39 days	Schwetz et al., 1973
New Zealand albino rabbit	male and female	dermal	275	12-22 days	Schwetz et al., 1973
Rhesus monkey	female	oral	<70 ug TCDD/kg	approx 4 weeks	McConnell, et al., 1978
Rhesus monkey	female	oral	3.5 ug TCDD/kg 0.013 ug TCDD/kg/day	28-44 weeks	Allen, et al., 1977
DBA/2J mice	male	oral	2570	30 days	Chapman and Schiller, 1985
$B6D2_1/J$ mice	male	oral	296	30 days	Chapman and Schiller, 1985
C57BL/6J mice	male	oral	182	30 days	Chapman and Schiller, 1985
C57BL/6J ($Ah^{b/b}$)	male	oral	159	22 days	Birnbaum, et al., 1990
C57BL/6J ($Ah^{d/d}$)	male	oral	3351	22 days	Birnbaum, et al., 1990
C57BL mice	male	oral	114	15-24 days	Vos et al., 1973

of 1.3 to 1.8 ug TCDD/kg (DeCaprio et al., 1986). Although this phenomenon has not been fully evaluated, female animals in general appear to be less sensitive to the acute toxicity of TCDD than do the males. This is exemplified by female guinea pigs and Sherman rats which were found to be less sensitive to the lethal effects of TCDD than the male animals (Table II). The magnitude of the response may vary across or within species, but there are a number of consistent lesions. At the measured LD_{50}, in all animals examined, there is a species specific delay in onset of the appearance of toxicity, generally about three weeks. Animals begin to lose weight immediately and continue to lose up to 50% of their body weight, depleting their adipose tissue until their death. This is characterized as the wasting syndrome. Body weight loss was also observed after a single TCDD treatment in male Fischer F-344 rats (po 100 ug TCDD/kg), male albino guinea pigs (ip 2 ug TCDD/kg) and male C57BL/6 mice (po 360 ug TCDD/kg). Based on observations of food consumption of these animals and pair fed animals, Kelling et al. (1985) concluded that hypophagia appeared to cause some loss of adipose tissue accounting for weight loss. The precise mechanism underlying the wasting syndrome is not known however, body weight loss appears to contribute more to lethality in the some species and strains (ie., guinea pig and the Sprague Dawley rats than in others (ie., Fischer rats and C57Bl/6 mice) (Kelling et al., 1985; Peterson, et al.,1984).

III. SYSTEMIC EFFECTS

A. INTRODUCTION

Administration of TCDD has consistently been shown to cause a variety of toxic effects. Most pathological changes were found to affect epithelial tissue; however, specific dermal lesions are observed in some species. In man, exposure to TCDD results in chloracne, a skin lesion, which

is characterized as "follicular hyperkeratosis in the absence of imflammatory comedones"[3] In monkeys there is hair loss along with thickening and keratinization of glandular tissue. Oral administration in monkeys, rabbits, and hairless mice results in dermal lesions. Gastrointestinal lesions characterized as hyperplasia of the gastric mucous or intestinal epithelium have been found in monkeys, cows and hamsters but not in rats, and guinea pigs. Urinary tract hyperplasia has been reported in the guinea pig, monkey and cow. Liver toxicity, immunotoxicity, and thymic atrophy occur in several species at very low doses. In addition, tissue specific fetal development is affected by TCDD in several species (Poland and Knutson, 1982). These effects occur at low doses and some dose-response relationships have been established for a few of these endpoints. The comparison of dose response of several endpoints is the focus of this report. In all mammalian species tested thymic atrophy is one of the most sensitive clinical indicators of toxicity.

B. THYMIC ATROPHY

Guinea pigs are most sensitive to the acute lethal effects and to may of the systemic effects of TCDD. Harris et al. (1973) administered eight weekly doses of 0.0, 0.008, 0.04, or 0.2 ug TCDD/kg to female guinea pigs which resulted in a statistically significant[4] decrease in thymus weight in the 0.04 ug TCDD/kg dose group (LOAEL = 0.0057 ug TCDD/kg/day equivalent dose, NOAEL = 0.0011 ug TCDD/kg/day). The Michaelis-Menten functional fit of this data (Graph A1)[5] is poor primarily because of the minimal number of data points. Additional dose-response

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[3]Zugerman, C. (1990) Chloracne Clinical Manifestation and Etiology*Dermatologic Clinics* 8(1), 209.

[4]All statistical significance described in this report was determined by the study investigators.

[5] All graphs discussed in text are located at the end of the document.

analysis was not performed on this data set. In A 90 day study, Decaprio et al. (1986) administered 2, 10, 76, and 430 ppt TCDD in the feed. Daily intake was calculated as 0.00012, 0.00061, 0.0049, and 0.026 ug TCDD/kg/day in males and 0.00012, 0.00068, 0.0049, and 0.031 ug TCDD/kg/day in females. In males, the more sensitive species in this study, the absolute and relative thymus weight was significantly different from controls at the 0.0049 ug TCDD/kg/day (See Table III). This level is comparable to that seen in the Harris et al. (1973) study.

In female rats, 31 daily doses of 0.0, 0.1, 1.0, and 10 ug TCDD/kg resulted in a decrease thymus weight after 24 days in the 0.1 ug TCDD/kg/day dose group (cumulative dose 0.24 ug/kg)(Harris, et al., 1973). In a 13 week study Kociba et. al., (1976) demonstrated a dose related decrease in thymus weight in male and female Sprague-Dawley (S-D) rats administered 0.1 ug TCDD/kg/day orally 5 days/week. Graphic depiction of this percent decrease in thymic weight in male and females (Graph A2) demonstrates a "good fit" to the Michaelis-Menten type response. The female Sprague Dawley rats appeared slightly more sensitive to this response, although the doses where a decrease in thymic weight was observed was the same. Microscopic examination revealed complete involution of the cortical region of the thymus of rats administered 1.0 ug/kg/day and slight involution in those administered 0.1 ug TCDD/kg/day. The statistically significant decrease in thymus weight appeared to be a sensitive indicator of microscopic changes in the thymus. A dose-dependent decrease in absolute thymus weight was reported in Rhesus monkeys administered acute oral doses of 70 ug TCDD/kg (lowest dose administered) or greater (McConnell, 1978). However, this dose may not accurately reflect the sensitivity of this endpoint in the monkey, but does provide information on the consistency of this response.

Thymic atrophy has been reported in rats, guinea pigs, monkeys, hamsters and there are

suggestions of thymic effects in humans. Effects on thymic hormones have been observed in humans exposed (not quantified) to TCDD. A statistically significant lower mean serum thymosin $_{alpha\text{-}1}$ was observed in a study that compared 94 people allegedly exposed to TCDD in contaminated residential areas to a nominally unexposed control population. The study found thymosin levels were inversely associated with years of living in the contaminated area (up to 7 years) when controlling for age and socioeconomic factors. Thymosin is produced in the epithelioid cells of the thymus and functions to modulate the maturation of prothrombocytes to mature thrombocytes. Decreased serum thymosin $_{alpha\text{-}1}$ suggests an effect on the thymus in this human population exposed to environmental levels of TCDD. Unfortunately concentrations of TCDD in these areas not serum levels (a more accurate estimate of TCDD exposure) were reported (Stehr-Green et al., 1989).

In six studies a range of single doses of TCDD were administered orally to C57BL/6J mice. Four of these studies (McConnell et al., 1978a, Birnbaum, et al., 1990, Chapman and Schiller, 1985, and Bombick et al., 1988) tested doses that were greater than 30 ug TCDD/kg and found a statistically significant decrease in thymic weight or thymic atrophy at all doses tested. Vos, et al., (1974) administered single oral doses of 0, 0.2, 1.0, 5.0, and 25 ug TCDD/kg/day. At 1.0 there was a statistically significant decrease in thymus/bwt, with a NOAEL at 0.2 ug TCDD/kg. Kerkvliet and Brauner (1990) tested similar doses; 0, 0.2, 1, 2.0, and 5.0 ug TCDD/kg, in female C57BL/6J mice reporting a dose-dependent decrease in relative thymic weight. Graphic comparison of these two studies (Graph B1) demonstrates similar responses in the 1 to 10 ug/kg dose range, although the male mice of a different strain appear more sensitive. Since the linear range of the dose response curve lies between 0.2-10 ug TCDD/kg a linear model was also fit to this range therefore data sets could be directly compared. [Graph B2 demonstrates that the slope of both these dose-

response curves are similar (Vos, male mice = 0.57 and Kerkvliet, female mice = 0.46)]. It becomes apparent when comparing this Graph B2-(A) to Graph B2-(B) that the range of doses used for comparison is important. Although many studies describe thymic atrophy, few have enough data to plot comparative dose-response curves, therefore removing data points for this type of comparison will generally be problematic.

Poland and Glover (1980) found a dose-dependent decrease (graphically depicted) in thymic atrophy in C57BL/6J and DBA mice after a single i.p. injection. The ED_{50} was 10^{-8} mol/kg(3.22 ug TCDD/kg) in the C57BL/6J mice and the DBA/2J mice were 10 X less sensitive to this effect. The ED_{50} for induction of the Aryl Hydrocarbon Hydroxylase (AHH) in these animals was one-tenth the ED_{50} for thymic atrophy in both DBA and C57BL/6J mice.

In a review of the dose-response literature it is critical to note that the intra- and inter-species sensitivities are linked to the Ah locus (Whitlock, 1987). Different responses for TCDD in a variety of strains of mice were investigated to determine if the toxicity segregated with the Ah responsive mice. Thymic atrophy was observed after a single i.p. injection of 20 ug TCDD/kg TCDD was administered to sensitive species C57BL, C3H, and BALB mice. The same dose produced no thymic atrophy in DDD, AKR, and DBA mice (less sensitive species). These effects corresponded well with the induction of aryl hydrocarbon hydroxylase (AHH) activity in the livers of these strains of mice. The toxicity of TCDD to the thymus segregates with the Ah locus. (Nagayama, et al., 1989) Intraspecies differences do not appear to be related to different levels of cytosolic Ah receptor, but to a different cellular response to the ligand-receptor complex. Bombick et. al., (1988) administered multiple doses of TCDD in a single i.p. injection to DBA and C57BL/6J mice. The LOAEL for thymic atrophy in the DBA (115 ug TCDD/kg) was four times greater than

the LOAEL (30 ug TCDD/kg lowest dose tested) in the C57BL/6J mice. A comparison of thymic atrophy in wild type (sensitive) and congenic strains of C57BL/6J by Birnbaum, et al., (1990) indicated that the sensitivity of these strains is associated with the Ah locus.

Four strains of mice with differing susceptibility to Ah receptor mediated toxicity were used to investigate the role of this receptor in thymic atrophy and related immunotoxicity. C57BL/6, DBA, C3H/HEN, B6D2F1 male mice were administered a single i.p. injection of 0, 1.2, 6.0, and 30 ug TCDD/kg TCDD. All but the DBA mice showed a statistically significant decrease in thymic weight at the two highest doses. The dose-response relationships using a "Michaelis-Menten" type fit (See table III and Graph B3) demonstrate a good correlation between receptor induction and thymic atrophy (Vecchi et al., 1983). The fit to some of the data sets was not satisfactory for the C57BL/6 and DBA mice, therefore the use of other functions was explored. These strain sensitivities were are also demonstrated with the Power law function (Graph B4) with good correlation coefficients in the responsive strains, 0.84 to 0.97. Linear fits to these data can also be used to demonstrate relative sensitivity, however correlation coefficients are generally lower (Graph B5) resulting in a poor fit.

The comparison of Long Evans (LE) and Han/Wistar (H/W) rats provide a unique opportunity to observe intraspecies differences. Long Evans rats are more sensitive to the acute toxic effects of TCDD than Han/Wistar rats, differences which not are believed to correspond with the Ah locus. L-E and H/W rats were administered a single i.p. dose of 5, 50, and H/W rats were additionally administered 500 ug TCDD/kg. Tissues were weighed at day 1, 4, 8, 16, and 32. A decrease in thymus to body weight ratio was observed 8 days post dosing in H/W rats at a dose of 5 ug TCDD/kg. No effect was seen at this dose on day 1 or 4 post dosing. In L-E rats this

same dose produced a decreased thymus to body weight ratio, but was observed earlier at 4 days post dosing. (Pohjanvirta, et al., 1989, 1990)

The distribution of TCDD in these two strains of rats was also examined by Pohjanvirta, et al., (1990). Both strains were administrated a single i.p. injection of 5 ug/kg ^{14}C-TCDD. Although many tissues were examined, liver, thyroid, kidney, spleen, and lung had very similar distribution patterns across tissues, but the distribution of TCDD to the thymus was very different. In Long Evans rats there is a continuous increase in TCDD concentration from 4 hrs to 16 days to a maximum of 0.18% of total dose concentrated in the thymus, followed by a decrease at the 32 day endpoint. In the Han/Wistar rat the concentration of TCDD was about half the level in the L-E rat (0.07%), and remained constant from day 1 to day 8, followed by a decrease (Pohjanvirta et al., 1990). Further studies are needed to explain the differences in tissue distribution and TCDD sensitivity in these two strains of rats.

Olson et al., (1980) evaluated the toxicity of TCDD in the Golden Syrian Hamster, a species not highly sensitive to the effects of TCDD having a 50 day LD_{50} of greater than 3000 ug TCDD/kg, ip ; and an oral LD_{50} of 1157 ug TCDD/kg. Olson et al. (1980) also evaluated the thymic response to TCDD after a single ip injection of 0, 5.0, 25, 100, 500, 1000, 2000, and 3000 ug TCDD/kg. Because of the number and range of data points, these data were evaluated more extensively using all the functional forms described in the introduction. Graph B6 depicts a "Michaelis-Menten" dose-response relationship, and a dose-dependent decrease in thymic weight was noted at 100 ug TCDD/kg which becomes significant (see Table III) at 500 ug TCDD/kg/day and greater. Reduced thymus weight was the most sensitive alteration noted in gross and histopathological examination. Olson et. al.(1980a) measured tissue distribution of radioactive

TCDD in the hamster after a single oral dose of 650 ug TCDD/kg. The distribution to the thymus reached a maximum at day 1 post injection and by day 3 TCDD content (measured as percent dose TCDD/g tissue) had begun to decrease from the thymus. The peak concentration in other tissues varied from day 1 to day 10 (liver was day 3) and had decreased substantially in all tissues by day 20. An important factor in tissue specificity may be excretion and distribution rates.

The Olson et al. (1980) data set (Table III) was used for additional dose-response analysis because of the number of data points in this study. In this data set, hamsters were administered single oral doses from 5 to 3000 ug TCDD/kg and thymus weight was measured at 50 days post-dosing. The percent response data were evaluated using the Linear, Michaelis-Menten and Hill functional forms described in the introduction. These data normalized as percent response provided a better fit to the functions than raw data. This could be due to the imprecision of changes in tissue weight as a toxic endpoint, or that normalized tissue weight is a more biologically relevant indicator of toxicity. Because negative values of percent response are not compatible with the Power Law function, raw data was used when evaluating this function. The linear model of normalized response (Graph B6-(A) did not fit well ($R^2 = 0.65$) presumably because it not reflect the biology of receptor-ligand interactions. The graphic representation of these data using the Michaelis-Menten function does begin to show a maximum response through the top dose of 3000 ug TCDD/kg (Graph B6-(B) and appears to reflect biological phenomenon. The third functional form used was the Hill plot (graph B6-(C) which also incorporated the data at the high doses. The log-linear plot of the Hill function depicts an "inertial" type of response at low doses with a "g" value over the entire dose range of 1.09, a linear/superlinear dose response. The Power Law function (Graph B-7(A) graphed over the entire dose range demonstrated a sublinear (g = -0.39,

$R^2 = 0.76$) decrease in absolute thymus weight. The Power Law function was subsequently applied systematically to subsets of doses to obtain optimal R^2 values. The Michaelis-Menten and Hill plots could not be used for this additional analysis because of the restrictions within these functions. The result of this exercise was to graphically observe a change in kinetic order from the low dose, 5.0 to 500 ug TCDD/kg, (Graph B-7(B) with a "g" value of -0.17 ($R^2 = .85$) to the high dose data, 500 to 2000 ug TCDD/kg (Graph B7-(C) with a "g"value of -1.57 ($R^2 = .99$). This increase in kinetic order with increasing doses suggests an inertial response at low doses and a synergistic response occurring at high doses suggesting that an inhibitory protein may be saturated at higher doses or induction of additional factors may occur at high doses to enhance the response. However, this is very speculative due to the uncertainties inherent in the use of clinical endpoints and reported means values, the constraints of using % response normalized data, and the limitations of evaluating a single species.

Several factors including tissue distribution, nuclear uptake, receptor concentration, and receptor affinity, could also be responsible for the differences in toxicity observed in hamsters, rats guinea pigs and mice. Pohjanvirta et al., (1990) identified strain differences in tissue distribution, Lund et al., (1982) found that nuclear uptake of TCDD in the thymus is only 6% of the uptake in the liver, and Carstedt-Duke (1979) studied the distribution of the TCDD receptor in various tissues in the Sprague-Dawley rat. The highest concentration of receptor, in tissues examined, was found in the thymus 25.2 fmol/mg protein. Cultures of several tissues from the Sprague Dawley rat were incubated with 10 nM TCDD to determine cytosolic Ah receptor concentrations. In the thymus, receptor concentrations remained relatively constant between day 2 and day 70 (Gasiewicz, 1985). Although species differences in these factors are not well characterized the differences seen

in tissues provide evidence that these factors are also involved in species differences.

Studies indicate that thymus cells have a high concentration of Ah receptors, and that TCDD induces receptor mediated enzyme induction in this tissue (Cook et. al., 1987). Dose related enzyme induction (7-ethoxycoumarin O-deethylase activity) has been observed in human thymic epithelial (HTE) cultures with a NOAEL of 0.1 nM and LOAEL of 1 nM. In HTE cultures it was observed that individual cultures differ in their sensitivity and that the Ah receptor concentration is not an accurate predictor of enzyme activity. In addition, in HTE cultures there is an Ah receptor mediated decrease in thymocyte maturation. This was determined by co-culturing thymocytes with HTE and examining the responsiveness of these cultures to mitogens (concanavlin A and phytohemagglutinin). Concentrations of TCDD suppressing thymocyte maturation ranged from 0.1 nM to 1.0 nM. (Cook et. al., 1987)

The pathology of TCDD induced thymic atrophy has been characterized as a depletion of small cortical cells in the thymus, thinning cortex and an increase in macrophage in the cortex characteristic of cell damage (McConnell, et al., 1978a). Kociba found in a 13-week repeated dose study in rats, a pronounced decrease in thymocyte number in the cortical region of the thymus (Kociba, et al., 1976)

Thymocytes develop into mature T-lymphocytes, and apoptosis occurs as a natural part of the selection process in this maturation. TCDD and glucocorticoids both increase thymocyte apoptosis. This could have a dramatic effect on the immunological status of the animal (Nebert et al., 1990). McConkey and Orrenius (1989) proposed that the decreased number of thymocytes seen in vivo experiments results from an increase in Ca^{2+}-mediated endonucleases, normally seen as part of the thymocyte maturation process. It has been suggested that this effect is similar to the

effect seen with exogenous glucocorticoid treatment. Analogies in the mechanisms of action have been noted between glucocorticoid and TCDD nuclear receptor mediated mechanisms of action. Similarity of their action on thymocyte Ca^{+2} endonucleases as measured by %DNA fragmentation led researchers to propose a receptor mediated mode of action on thymocytes. Additional evidence that TCDD and glucocorticoids act in a similar manner to increase thymic atrophy through depletion of thymocytes includes the findings (McConkey and Orrenius 1989) that inhibitors of glucocorticoid induced thymic suicide, reduced the effects of TCDD, and reduced the glucocorticoid sensitivity in thymocytes. This does not preclude the possibility of different mechanisms of action with shared modulators of receptor-ligand interaction.

An alternative mechanism to induce thymic atrophy was proposed by Bombick, et al., 1988. After administration of TCDD to sensitive mice an increase in protein-tyrosine kinase was observed. Previous reports (Bombick et al., 1988) found that protein tyrosine kinases are associated with lymphocytes, and that they may be involved in lymphocyte maturation. This seems very plausible especially since the protein kinase p60 activates oncogenes and may play a role in mitogen signal transduction. Hence, any inhibitor of mitogenesis and signal transduction initiated by TCDD on the DNA would stop maturation and produce thymic atrophy. A dose-dependent increase in protein kinase was observed following *in vitro* exposure of 2-10 nM TCDD to B lymphocytes. The increase occurred rapidly (increases observed 5 minutes after exposure) and was significant at approximately 2.5 nM (Clark et al. , 1991). The significance of this observation to TCDD mediated immune alterations *in vivo* has not been determined.

Table III Species Comparison of Thymus Weight Changes After TCDD Administration

Species	Dosing Regimen	Effect	Dose ug TCDD/kg	Response % change Thymus Weight				NOAEL	LOAEL	Reference
DBA/2J	single i p (9.1 corn oil/acetone)	decrease thymus weight (day 2)	0 30 60 115	0 4.7% 10 6% 40 1%*					115 ug /kg	Bombick, et al , 1988
C57BL/6J female mice	single oral	decrease thymic/bwt 7 days post dosing and absolute thymus weight	0 0.2 1.0 2 0 5.0	0 thymus/bwt +3% -6.6 % -16.7 % -23.4%		0 +4 5 % -5 1 % -12.9 % -19.9 %				Kerkvliet and Brauner, 1990
C57BL/6J	single i.p. (9.1 corn oil/acetone)	decrease thymus weight (day 2)	0 30 60 115	0% 27.2%* 35.0%* 53.1%*					30 ug/kg ≈ 0.92 nM/kg	Bombick, et al., 1988
C57BL/6 Schst. male	single oral	decrease thymus weight (week 2 and week 6)	0 0.2 1.0 5 0 25.0	0% 9.3% 12.5%* 33.1%* 79.9%*		0% 3 5% 18.4%* 45 6%* 79.8%*			1.0 ug/kg ≈ 3.1 nM/kg	Vos, et al , 1974
C57BL/6 DBA C3H/HeN B6DF1 male mice	single i.p. injection measurements 12 days post dosing	% decrease thymus weight	0 ug/kg 1.2 6.0 30.0	C57BL/6 0 % 11 % 44 %* 54 %*	DBA/2 0 % 19 % 16 % 27 %	C3H/HeN 0 % 20 % 40 %* 27 %*	B6D2F1 0 % 8 % 24 %* 44 %*	1.2 ug/kg C57BL, C3H, B6	6.0 ug/kg C57BL C3H B6D2F1	Vecchi et al., 1983
Long Evans rats males	single i p	decrease thymus weight	0 5.0 50.0	0 Day 4 20%* 20%*	0 Day 8 40%* 60%*		Day 16			Pojhanvirta, et al., 1989
Sprague Dawley rats males	oral 5 X week 13 weeks	decreased thymus weight	0 (0) (tot. dose 0.001 (0.065) 0.01 (0 65) 0.1 (6.5) 1.0 (65)	0 1.9% 3.8% 21.0% 75 0%						Kociba, et al., 1976
Sprague Dawley rats female	oral 5 X week 13 weeks	decreased thymus weight	0 (0) (tot dose) 0.001 (0.065) 0.01 (0.65) 0.1 (6.5) 1.0 (65)	0 7.5% 15 0% 42.5% 77.5%						Kociba, et al., 1976
Golden Syrian Hamster male	single ip injection measure 50 days post-dosing	decrease thymus weight	0 5 25 100 500 1000 2000 3000	0 0 1.9% 2.9% increase 32.7% 53.5%* 84.2%* 94.0%* 83.2%		0 101 g (raw data) 0.099 g 0.104 0.068 0 047* 0.016 0 006 0 017			500 ug/kg ≈ 155.0 nM/kg	Olson, et al , 1980
Guinea pigs Hartley	continuous dose in feed 90 days	decrease relative thymus weight	Male Female 0 ng/kg/day 0 0.12 0 12 0.61 0.68 4 90 4.90 26 31	Males 0 % 15% 13% 24%*		Females 0% 6% 16% 12%				DeCaprio, et al., 1986
Guinea pigs Hartley female	8 X weekly oral	decrease thymus weight (day 56)	ug/kg/week (total dose) 0 0 0 008 (0 064 total) 0.04 (0.32 total) 0 2 (1 6 total)	0 17.8% 25 4%* 47 2%*					0.04 ug/kg ≈ 0 12 nM/kg (0.32 total ≈ 0 96 nM/kg)	Harris, et al., 1973

The development of mature thymocytes involves the passage of cells through a variety of subpopulations with unique phenotypes. Antigenic determinants can be used to identify these subpopulations. Kerkvliet and Brauner (1990) used flow cytometric analysis to quantify these alteration in thymocyte subpopulations after p.o. administration of 2 ug TCDD/kg in C57BL/6J female mice. There was a significant TCDD dependent increase in the percentage of $CD4^-CD8^-$ double negative (DN) thymocyte phenotypes and a decrease in the percentage of double positive (DP) $CD4^+CD8^+$ thymocytes. These changes were not observed in the lymphocyte population of the spleen indicating that the T-cell population of the thymus is uniquely sensitive to TCDD toxicity. Although dose-response trends were not reported in this study, the alteration in the percent of thymocyte subpopulations was a sensitive indicator of TCDD immunotoxicity. The alteration of these subpopulations has also been demonstrated *in vitro* further supporting the hypothesis that an imbalance in percent of cell types may be responsible for TCDD induced alterations in immunocompetence (Lundberg et al. 1990).

Fine, et al.(1990) investigated the effects of TCDD on the intrathymic development of T-cells by quantifying the effects of a single i.p. injection TCDD on subpopulations of cells in BALB male mice. All populations of thymocytes were reduced substantially, 50-76% decrease in total number of cells of each type, in addition the total cell population as measured by thymic cellularity was decreased approximately 90%. Although the total number and type of cells decreased the percent of cells in each population changed. This suggests that TCDD is working through two different mechanisms. There was an increase in the percent of CD4-CD8- double negative (DN) , CD4+CD8-, and CD8+CD4- single positive (SP) thymocytes cell types, and a decrease in

CD4+CD8+ double positive (DP) cell types. These data are supported by Baylock et al. (1992) who found that fetal thymic cell population percentages varied in a similar manner. They suggested that TCDD altered the maturation of fetal thymocytes affecting the transition from DN to DP. Holladay, et al. 1991) found the effect of TCDD on fetal thymocyte maturation was more significant at gestation day (GD)18 than at day 6 or day 14 after birth, indicating a sensitivity in fetal thymocytes that decreases with age (Blaylock et al., 1992).

Decreased thymus weight in the perinatal period is related to a decrease in immune system function, and as such may be of critical importance in the understanding of the toxicity of TCDD. Pregnant Wistar rats were administered a single oral dose of 0.0, 0.005, 0.5, 0.125, 0.25, and 0.35 ug TCDD/kg on gestation day (GD) 16. A dose related decrease in pup thymus weight was reported. In addition, there was a dose related increase in 7-ethoxycoumarin deethylase activity, and on biphenyl 2-hydroxylase activity at the above administrated doses, with a NOAEL for enzyme induction of 0.005 ug TCDD/kg (Madsen, et al., 1989).

TCDD produces a consistent response of thymic atrophy in the guinea pig, rat, hamster, monkey and in the sensitive mice. This effect is receptor mediated and although the mechanism is not completely understood, several plausible mechanisms have been proposed. Because the thymus is a key organ in immunological responses it is not surprising that TCDD affects the immune system.

C. Immunotoxicity

TCDD-mediated immunotoxicity and the implications of enhanced and suppressed immune function are described in detail in the Immunotoxicity chapter of this document (Kerkvleit and

Burleson 1992). In addition, detailed evidence that the immunotoxicity of TCDD and related halogenated aromatic hydrocarbons is, at least in part, Ah-receptor mediated is presented. Studies with multiple doses and protocols that most resembled other studies (similar species, strains and dosing regimens) were evaluated for comparison of immunological endpoints. Because of the variety of protocols tested to evaluate TCDD immunotoxicity some endpoints have not shown clear dose-response relationships, however there are some that do. The premise that immunotoxicity is at some level Ah receptor mediated leads to the assumption that the response of some immunotoxicity endpoints may be related to the response of other Ah receptor mediated events.

Exposure to TCDD results in thymic atrophy, alterations in bone marrow and general immunosuppression in almost every species examined. (Luster et al., 1979,1980) Suppression of cell-mediated immunity, (CMI), measured as a response to phytohemagglutin (PHA) and concanavalin A (Con A), may be observed in the neonate during thymic organogenesis; the most sensitive time for this type of immunotoxicity (Luster, et al., 1987). Vos (1974a) investigated the role of maternal treatment of (0, 1.0, 5.0 ug TCDD/kg) administered prenatally GD 11 and GD 18, and/or postnatally day 7 or 14 on cell-mediated immunity (CMI) of Fischer-344 rat pups. The response of spleen cells to PHA was significantly decreased in 25 day old pups after maternal postnatal exposure only at the 5 ug TCDD/kg level. However, after pre and postnatal maternal exposure there was a significant decrease in the PHA response at 1.0 ug TCDD/kg indicating that prenatal exposure is a more sensitive period for immunotoxicity. There was significant fetal toxicity associated with immunosuppression at the 5 ug TCDD/kg level. The susceptibility of pups is believed to be greater than in adult rats because of the role the thymus plays in CMI. Although the relevance of the thymic atrophy seen in animals as it relates to the adult human population may

be questioned, the thymus is an important organ in the development and function of the human immune system. Similar results were reported by Faith and Moore (1977) in thymus cells from 25 day old female Fischer rat pups exposed postnatally to TCDD. There was a significant decrease in responsiveness to PHA at the 5 ug TCDD/kg level, but not to Con A. The impact of pup age on CMI is assessed by comparing the responsiveness to PHA in 1 and 4 month old mice. The responsiveness to PHA was significantly reduced in 1 month old mice that were administered 4 weekly doses of 25 ug TCDD/kg TCDD. Four month old mice administered the same doses (0, 1.0, 5.0, and 25 ug TCDD/kg) weekly for 6 weeks had no differences in PHA responsiveness when compared to controls although all doses showed significant thymic atrophy. The suppression of cell mediated immunity in sexually mature (young adult) rats and mice was demonstrated to be a relatively insensitive endpoint which differed significantly from controls at the 25 ug TCDD/kg dosage level only. The difference in sensitivity based on age is consistent with what is known about the development of the thymus (Vos and Moore 1974a).

Thomas and Hindsdill (1979) described a dose-related decrease in relative thymus weight in offspring of adult female Swiss mice exposed to TCDD in feed at concentrations of 1, 2.5, 5 and 10 ppb for 4 weeks prior to mating. A statistically significant decrease in relative thymus weight and number of SRBC (sheep red blood cells) plaque forming cells per gram of spleen (both indicators of immunotoxicity) were observed in the offspring of animals exposed to 2.5 or 5 ppb TCDD. Although food consumption data was not reported in this study and daily intake and fetal dose can not be determined, the spectrum of toxic effects presented are relevant. Both endpoints are indicators of immunotoxicity. No overt signs of maternal toxicity were observed at doses producing immunotoxicity.

Another effect observed after prenatal exposure was that allograft rejection times were prolonged in a dose-dependent manner in 23 day old C57BL/6J mice exposed pre and postnatally (GD 14 and GD 17) to 2.0 and 5.0 ug TCDD/kg levels of TCDD (see Table IV) and in rats exposed postnatally on day 1,8, and 15 to the 5.0 ug TCDD/kg level (Vos and Moore 1974a).

Vecchi et al. (1980) examined the response of C57Bl/6J adult mice splenocytes to Con A and lipopolysaccharide (LPS) after TCDD treatment to assess cell-mediated immunity. Male mice were administered 0, 1.2, 6 and 30 ug TCDD/kg. At 7 and 14 days post dosing, splenocytes from these animals were tested for their response to Con A and LPS. At 30 ug TCDD/kg a significant increase in mitogenic response was observed at 1.6 and 3.2 ug/sample Con A, NOAEL=6.0 ug TCDD/kg. Their was no splenocyte response to LPS in mice administered 30 ug TCDD/kg. This study indicated that relatively high doses (30 ug TCDD/kg) are required to affect cell-mediated immunity in adults.

Vos et al. (1973) also examined cell-mediated immunity in female Hartley guinea pigs receiving 8 weekly doses of 0, 0.008, 0.04, 0.2, and 1.0 ug TCDD/kg and in female CD rats administered 6 weekly doses of 0, 0.2, 1.0 and 5.0 ug TCDD/kg (Table IV). Vos et al. (1973) selected delayed hypersensitivity to tuberculin toxin in guinea pigs and rats to assess CMI. The results are reported as diameter of skin reaction. In guinea pigs administered TCDD, sensitized with tuberculin on day 35 post dosing and challenged on day 47 and 54, there was a dose dependent decrease in diameter of skin reaction which was significant at the 0.04 ug TCDD/kg/week level (0.32 ug TCDD total dose). TCDD inhibited the delayed hypersensitivity response in these animals. There was no significant change in diameter of skin reaction in rats

sensitized on day 28 and challenged on day 42 (see Table IV). This response is consistent with results of acute toxicity data that found guinea pigs are more sensitive to the toxic effects of TCDD than rats. However, rats are generally not used for delayed hypersensitivity testing. Hence, the guinea pig results stand on their own.

Another method Vos et al. (1973) used to assess cell-mediated immunity is to examine local graft vs. host reactions (GVH). B6D2F1 recipient mice administered 0.0, 0.02, 1.0, and 5.0 ug TCDD/kg were injected with C57BL/6J mice spleen cells. To assess the GVH response the ratio of the weight of the right and left popliteal lymph nodes was compared. A dose dependent decrease in ratio was observed and was significant at 5.0 ug TCDD/kg (Table IV)

Rosenthal et al. (1989) assessed the response to endotoxin (E. coli LPS, 0.25, 5 and 25 mg/kg) administered 7 days post TCDD treatment to female B6C3F1 at single oral doses 0, 50, 100, and 200 ug TCDD/kg. Effects observed included an increase in mortality from 5 mg/kg endotoxin at the 200 ug TCDD/kg level and a decrease in the ability of mice administered 50 ug TCDD/kg to clear 2.5 mg/kg endotoxin from their blood. The sensitivity of this endpoint was not tested, as all administered doses resulted in an some effect (Table IV). The increased responsiveness to endotoxin segregated with the $Ah^{b/b}$ locus (sensitive strain) and was not seen in the $Ah^{d/d}$ (less sensitive strain) congenic mice. Furthermore, the response to endotoxin and TCDD was reversed by administration of methyl prednisolone leading the authors to conclude that an inflammatory component is involved in the response.

The most sensitive indicator of cell-mediated immunity is the reduction in cytotoxic T-lymphocytes (CTL). Nagarkatti et al.(1984) demonstrated a decrease in CTL response at a

cumulative dose of 4 ng/kg. Kerkvliet et al. (1990a) noted a dose-related decrease in CTL at 10 and 20 ug TCDD/kg (single oral dose TCDD). Graphic representation of there data indicated a dramatic decrease in the CTL response in C57BL/6J mice and a minimal decrease at these doses in non-responsive congenic mice. A controversial study by Clark et al. (1981) demonstrated a dose-related suppression of CTL in C57BL/6J mice. Doses as low as 0.004 ug TCDD/kg to 40 ug TCDD/kg resulted in a decrease in CTL activity. It has also been reported by Clark et al. (1981) that TCDD does not deplete the number of CTL precursors, but induces a population of suppressor T-cells. Clark's data demonstrate suppression at extremely low levels, however, the protocol used is very difficult to precisely replicate (Holsapple et al. 1991, 1991a).

Cell mediated immunity is only one type of response the body employs as a defense mechanism. Humoral immunity may be assessed by measuring the serum level of induced anti-toxin in response to toxoid challenge. Vos et al. (1973) measured the response to the tetanus toxoid in guinea pigs administered 8 weekly doses of 0, 0.008, 0.04, and 0.2 ug TCDD/kg. Guinea pigs were injected with toxoid on day 28 post TCDD administration and anti-toxin levels were measured 7, 21, and 28 days after initial injection. There was a significant increase in serum anti-toxin levels at 0.008 and 0.04 ug TCDD/kg levels 7 days after toxoid injection. however, not at 21 and 28 day. There was a significant decrease in anti-toxin levels in the 0.2 ug TCDD/kg dose at these two time points suggesting a dose-dependent response (Table V).

Another method of assessing humoral immunity is to quantify the production of specific antibodies in response to an antigen characterized as thymus-dependent and thymus-independent antigens. The sheep red blood cell (SRBC) antigen is the most common antigen used to assess immune competence after TCDD treatment. This assay has produced consistent and reproducible

results while using multiple doses. Therefore this endpoint will be evaluated more empirically. Vecchi (1980) assessed the immune response in C57Bl/6J male mice after i.p. treatment with 0, 1.2, 6, or 30 ug TCDD/kg. 4 X 10^8 SRBC, a T-dependent antigen, and 0.5 ug of type III pneumonococcal polysaccharide, a T-independent antigen, were used to assess humoral immunity. At all doses, with both antigens, and at all time periods assessed, TCDD significantly decreased the plaque forming cells/10^6 splenocytes (a measure of immune response) in a dose-dependent manner (Table V). The antibody-mediated immunity was suppressed in C57BL/6J mice at the lowest dose tested, 1.2 ug TCDD/kg, a dose lower than that found to suppress CMI.

Davis and Safe (1988) measured plaque forming cells (PFC)/10^6 splenocytes) in C57BL/6J mice after administration of 0.322, 0.422, 1.2, and 3.1 ug TCDD/kg followed by an i.p. injection of 4 X 10^8 SRBC. Although statistical significance was not assessed in this study, there was a dose-dependent decrease (Table VI) in PFC/10^6 viable cells seen in all dose levels. These authors also evaluated the response of other polychlorinated dioxin compounds to examine structure activity for Ah receptor affinity and concluded that the humoral response to T-dependent antigens is Ah receptor mediated. In addition the authors suggest that TCDD-receptor complex may modulate early B-cell differentiation.

Narasimhan et al. (1993) assessed the same endpoint in B6C3F1 mice and found a statistically significant decrease in PFC/10^6 viable cells at 0.05 ug TCDD/kg (Table VI). Analysis of dose-response for immunotoxicity is complicated by the variety of endpoints and dose ranges reported in the literature. However, the use of PFC/spleen was a consistent measure of humoral immunity in three studies. The Kerkvliet and Brauner (1990); Narasimhan et al.(1993) unpublished); Silkworth et al. (1989); and the Davis and Safe (1988) data sets were selected for

additional dose-response analysis. These data sets normalized to percent response, were fitted to the Michaelis-Menten function (graph C1) using doses in the same range[6]. All but the Kerkvliet and Brauner (1990) data set fit this function poorly. The fit to the Power Law function for all four data sets (graph C2) shows a consistent response between the Silkworth (DBA2) and the Kerkvliet data, ie, both fit the function well ($R^2 = 0.99$ and 0.98 respectively), and had comparable exponents in the function ("g" = 0.32, 0.30). The Davis and Safe (1988), Silkworth et al. (1989) and Narasimhan et al. (1993) data sets all had higher "g" exponents and poorer fits. A plausible explanation for these variation could be that test methodologies differed(ie. use of different control solutions) or there are genuine species and sex differences that modulate this response. It is interesting to note that the shape of the dose-response curves for the DBA and BALB mice used in the Silkworth et al. (1989) experiment are very different (Graph C2) and this suggests that the dose values used are capturing different segments of the dose-response curves for these two strains of mice.

The Narasimhan et al. (1993) data set was used for additional analysis because of the number and range of doses reported. The functions that best fit the raw data points were the Michaelis-Menten and the Hill functions (Graph C3-A, C3-B). The Power Law Function (Graph C4-A) fit all raw data values for PFC's/spleen with an R^2 value of 0.89 and a "g" value of -0.43. A Power Law functional fit of the entire data set using normalized percent response data could performed because at very low doses there was a negative value which is not mathematically

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[6]The entire data set from Safe, 1992 was not used for this analysis. Direct comparison to Kerkvliet and Brauner (1990) and Davis and Safe (1988) used data that from a similar dose range.

consistent with this function. Systematic selection and analysis of data subsets using % response normalized data (excluding extremely low values) resulted in values of "g" = 2.2; and $R^2 = 0.99$, in the low dose range (0.025 to 0.1 ug TCDD/kg) (Graph C4-B) suggesting superlinearity. In contrast, the high dose range (0.5-2.5 ug TCDD/kg) resulted in "g" = 0.23 and $R^2 = 0.87$, suggesting sublinearity or a lower rate of response (Graph C4-B). The higher exponent at the low dose may represent synergy or positive cooperativity. However it is also consistent with the existence of non-nuclear receptor-mediated interactions at low doses, or may suggest induction of additional inhibitory factor at higher doses. When an analysis of subsets of this data was applied to raw data a different pattern of dose-response was observed. At the low administered doses the absolute response resulted in a sub-linear fit with a "g" value of -0.57, $R^2 = 0.89$, whereas at higher doses a steeper linear fit was observed, "g" = -.99, $R^2 = 0.98$ (depicted in graph C4-C). Whether raw data of percent response normalized data is a better representation of low dose phenomenon can not be concluded from this analysis.

Kerkvliet et al. (1990) demonstrated differences in humoral immunity related to the Ah responsiveness of C57BL/6J congenic mice after acute exposure to TCDD. The dose-dependent decrease in splenic antibody response to the T-cell independent antigen TNP-LPS revealed differences in sensitivity which were consistent with strain Ah responsiveness. The response of these strains to T-cell dependent antigen was more complex and therefore could not be directly related to Ah receptor responsiveness (See Table V). Graph C5-A depicts the different sensitivities of the two strains using a Michaelis-Menten function with a reasonably good fit. The Power Law function also depicts strain sensitivity (Graph C5-B) although the fit is poor.

Vecchi et al. (1983) compared multiple doses (3) in three strains of mice to demonstrate that the decrease in antibody production as measured by PFC's/10^6 spleen cells segregated with the Ah receptor (See Table IV, Graph D1-A, D1-B). The Michaelis-Menten and the Power Law function are both good representations of the species differences in sensitivity. Because of the differences in the correlation and the "g" value in the Power Law, it appears that the best fit to this functional form is at the doses where a change in response is measured.

In an acute study (Morris et al. 1992) a dose-dependent decrease in anti-SRBC IgM AFC (antibody forming cells)/spleen was observed in all doses (4.2, 14, and 42 ug TCDD/kg) administered orally to the B6C3F1 mouse (Ah responsive strain) however, only at the 42 ug TCDD/kg dose in DBA/2J mice (less responsive). Subchronic administration of the same cumulative dose revealed statistically significant decrease (not clearly dose dependent) at all doses in both strains. This study clearly shows (Table V) that exposure conditions can alter response. The acute exposure study demonstrates that B6C3F1 mice are approximately 10 times more sensitive than the DBA mice, however the results of the subchronic study suggest that there may be a modification of the Ah mediated strain sensitivity. These results support what is known about Ah receptor mediated toxicity that cumulative doses will establish an equilibrium between receptor-ligand formation, receptor biosynthesis, and receptor-mediated effect (EROD induction, or suppressed immune response). A lower dose can maintain this equilibrium whereas a higher dose would be required to induce the same effects with a single dose.[7] Clearly Morris et al. (1992) has demonstrated this phenomenon with humoral immunity.

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[7]In comparing Birnbaum and DeVito's data (Table X) and Safe's data (Table IX) the dose to induce a statistically significant increase in EROD after acute administration was 0.5 ug/kg whereas administration of 0.0975 cumulative dose in a subchronic protocol induced the same level of EROD induction.

Kramer et al. (1987) using highly purified murine B-cells suggested early events may be the target of TCDD in these cells since TCDD increased basal kinase activity in the presence of added phospholipid, but did not increase protein kinase C (PKC)activity. The authors proposed that TCDD affects basal kinase activity at the level of protein phosphorylation. Hence, protein kinase involved in phosphorylation and early activation may be the target of TCDD and result in altered B-cell differentiation. Changes in basal protein kinase activity, although Ah receptor mediated, may not require translocation to the nucleus and therefore this endpoint may respond very differently than other events mediated through XRE activation.

It has been hypothesized that B-cells are a direct target of TCDD toxicity (Dooley and Holsapple 1988). The effect of TCDD on T- and B- cell interactions in the antibody response was investigated using a T-dependent antigen SRBC's and two T-independent antigens DNP-Ficoll (dinitrophenol-Ficoll) and TNP-LPS (trinitrophenol-lipopolysaccharide). The antibody response was assessed by enumeration of antibody forming cells (AFC's) in spleens of female B6C3F1 mice administered 0, 0.1, 1.0 and 10 ug TCDD/kg daily for 5 days (Table V). Five ug TCDD/kg total dose produced a decrease in AFC induced by all three antigens. Their findings that the response to T-dependent and T-independent antigens was decreased at the same dose level of TCDD led the authors to conclude that B-cells were the primary target of TCDD and that this immunosuppression is at the level of B-cell differentiation. These doses are high doses for immunomodulating effects (100 X the LOAEL in the Narasimhan et al. (1993) study) and therefore may not represent the lowest dose effect.

To assess the suppression of humoral immunity by TCDD and the role of the Ah receptor in that suppression, Holsapple et al. (1986), and House et al. (1990) administered a single p.o. dose

of 0, 0.05, 0.1, 0.5, 1.0, and 2.0 ug TCDD/kg to female B6C3F1 mice and subsequently administered SRBC a T-dependent antigen. The response was measured by the enumeration of IgM antibody forming cells (AFC) in the spleen. Significant dose-dependent reduction in IgM AFC was observed at 1.0 ug TCDD/kg (Table V).

The immune system is very complex with many interactions across its components. Susceptibility to infection may be affected by TCDD. Thigpen et al. (1975) found that C57BL/6J male mice treated with weekly doses of 0, 0.5, 1.0 and 5.0 ug TCDD/kg for 4 weeks (equivalent to 0.07, 0.14, and 0.7 ug TCDD/kg/day) and subsequently administered a challenge of *Salmonella bern* responded with a dose dependent increase in mortality at the 1.0 and 5.0 dosage levels. The increase in mortality was not seen after administration of a challenge dose of the virus *Herpesvirus suis*. Thigpen et al.(1975) attributes this to differences in pathogenesis of the infections. In this study, the administered doses of TCDD resulted in no clinical signs of toxicity; yet resulted in immune suppression. House et al.(1990) attempted to elucidate the effects of TCDD on a variety of immune system factors. Using the influenza virus to assess responses. Survival after administration of 0.1, 1.0, and 10 ug TCDD/kg was significantly decreased in a dose-dependent manner in all doses tested. In addition it was reported that TCDD did not effect interferon production; a system generally initiated by a viral infection.However, TCDD may directly interfere with the effector cells in this system by modulating the production of cytotoxic T-cells and natural killer cells (House et al. 1990). It was also reported that a dose-dependent increase in mortality in TCDD treated mice following an influenza challenge in which the LOAEL (lowest dose tested) was a single dose of 0.1 ug TCDD/kg (House et al. 1990)

Table IV — Cell-Mediated Immunity

Species	Dosing regimen	Effect	Dose (ug TCDD/kg) - Response (%change)				NOAEL	LOAEL	Reference
C57BL/6J 23 day old mice pups	0, 2.0, 5.0 ug/kg pre and postnatally GD 17 and 17 and day 1,8,15	increase rejection time of tail-skin graft	0 2.0 5 0	0 19%* 25%*					Vos and Moore, 1974a
F-344 28 day old rat pups	0 and 5 ug/kg maternal postnatally on days 0, 7,14	increase rejection time of tail-skin grafts	0 0 5.0	0 +18%*					Vos and Moore, 1974a
Female albino rats CD	0, 0.2, 1.0, 5.0 ug/kg weekly for 6 weeks.Day 28 rats were injected with M. tuberculosis and day 42 injected with 5 ug tuberculin PPD	Thickness of skin reaction on 24 and 48 hrs after final injection	0 0.2 1 0 5.0	Skin diameter 24 hrs 0 +3 4% +5.1% +0.7%	Skin diameter 48 hrs 0 -10 5% -5.9% -4.6%	Skin Thickness 24 hrs 0 -9.5% +6 62% -10.5%	Skin Thickness 48 hrs 0 -2 7% -8.2% -13%	N/A	N/A	Vos et al., 1973
Male B6D2F1 mice	0, 0.2,1.0 and 5.0 ug/kg weekly for 4 weeks C57BL mice. Recipient mice received graft of spleen cells in right hind foot pad.	Response measured as difference between right and left popliteal lymph node.	ug/kg (total dose ug/kg) 0 0 0 2 0.8 1.0 4.0 5 0 20.0	Right/left Popliteal lymph nodes % differs from control 0 -0.2% -38% -66%*				N/A	N/A	Vos et al , 1973
Female B6C3F1 mice	Single oral dose of 0, 50, 100 or 200 ug/kg followed by 7 day iv treatment with E.coli LPS endotoxin 5 or 25 mg/kg	Response mortality at 48 hrs	ug/kg 0 50 100 200	Mortality 5 ug/kg endotoxin 0% 0% 0% 87.5%*			Mortality 25 ug/kg endotoxin 0% 50%* 100%* 100%*			Rosenthal, et al., 1989
C57BL/6 male mice	weekly i.p. injection X 4 wks At week 5 sensitized painting ears with 3% oxazalone challenged with 1% oxazalone	ear thickness 10^2 mM measured	Dose 0 0.4 ug/kg 4 ug/kg 40 ug/kg	Increment in ear thickness 24 hrs after challenge 0 +54% -38%* -32%*				0.4 ug/kg	4 ug/kg	Clark, et al., 1981
Hartley guinea pigs	8 X weekly oral administration	day 35 0.05 ml injection of tuberculin bacillus hypersensitivity tested day 47 and 54 with tuberculin injections (1.25 ug) skin reactions measured 24 and 48 hrs after tuberculin injections skin diameter measurements % difference from control	Dose ug/kg/wk (total dose) 0 0.008(.064) 0.04 (0.32) 0.2 (1.6)	skin diamet er 47d-24hrs 0 -4% -8% -29%*	diameter skin 47d - 48 hrs 0 -9% -15%* -42%*	skin diameter 54d - 24 hrs 0 -5% -9%* -22%*	skin diameter 54d - 48 hrs 0 -9% -12%* -41%*			Vos, 1973

Table V Humoral (Antibody-Mediated) Immunity

Species	Dosing regimen	Effect	Dose (ug/kg) - Response (%change)				NOAEL	LOAEL	Reference
male C57BL/6J 6-8 wks	i.p with TCDD followed by (SRBC) in-jection PFC measured 7, 14 , 21,and 42 d.after TCDD	measured % decrease from control PFC/10^6 splenocytes	0 ug/kg 1.2 6 30	0 7d 54%* 86%* 96%*	0 14d 42%* 70%* 89%*	0 21d 60%* 77%* 89%*	0 42d 55%* 85%* 88%*	N/A	1.2 ug/kg	Vecchi, 1980
Hartley Guinea pigs female	8 X weekly oral administration	day 28 and day 42 0.1 ml tetanus toxoid injected into hind footpad Serum antitoxin measured day 35, 49, and 56.	0 dose ug/kg/wk 0.008 (.064) 0.04 (0.32) 0.2 (1.6)	0 Day 25 +16%* +21%* -5%	0 Day 49 -13% -22% -43%*	0 Day 56 +8% -7% -29%*		Day 35 N/A Day 49& 56 0.04	Day 35 0.008 Day 49 0 2 Day 56 0.2	Vos, 1973
B6C3F1 mice	oral administration 1 acute oral dose 2 14 day cumulative dose	decrease Formation of anti-SRBC IgM Antibody forming Cells	0 ug/kg 4.2 14 42	1. 0 64 % 67 5 % 83 %		2. 0 78% 75% 88%				Morris et al., 1992
DBA/2J mice	oral aministration 1. acute oral dose 2. 14 day cumulative dose	decrease Formation of anti-SRBC IgM antibody forming cells	0 ug/kg 4.2 14 42	1. 0 16 % 9.5 % 33 %		2. 0 56 % 83 % 88 %				Morris et al, 1992
Female B6C3F1 mice	oral administration of TCDD daily for 5 days 48 hrs after last treatment sensitized with antigen	Antibody forming cells measured after sensitizing with SRBC, DNP-Ficoll, TNP-LPS in vivo assay	0 ug/kg/day 0.1 1.0 10.0	0 Response SRBC -32% -72%* -94%*	0 Response DNP-Ficoll -60% -86%* -87%*	0 Response TNP-LPS N/A -64%* ND				Dooley and Holsapple, 1988
Female B6C3F1 mice	single p.o. TCDD, day 8 post dosing immunized with SRBC.	Spleen cell IgM anti-SRBC AFC response measured as change in IgM AFC /10^6 recovered cells	0 ug/kg 0.05 0 1 0.5 1.0 2.0	0 % change from control IgM AFC +4.2% +5% -26% -48%* -66%*		0 weight % change from control +18% -4% +12% +4% 6%		IgM AFC = 0.5	IgM AFC = 1.0	Holsapple, et al., 1986
C57BL/6J male mice	i.p. injection of TCDD weekly for 4 weeks bacterial and viral challenge 2 d.after dosing	mortality rates reported	ug/kg/week (ug/kg/day) 0 (0) 0.5 (0.07) 1.0 (0.14) 5 0 (0.7) 10 (1 4) 20 (2.8)	Bacterial challenge mortality 25% 25% 65% 70% 65% 95%		Viral Challenge 15% mortality ND ND 15% 0% 10%				Thigpen, et al., 1975
C57BL/6J male mice $Ah^{d/d}$, and $Ah^{b/b}$	single oral dose TCDD prior to ip injection of 25 ug TNP-LPS	% decrease number of nucleated spleen cells	0 ug/kg 1 5 10 20 50	0% ($Ah^{b/b}$) 10.6 % 17 % 28.5 % 24 % not tested		0 % ($Ah^{d/d}$) not tested 12.6 % 22.1 % 15.1 % 25.2 %		1 ug/kg $Ah^{b/b}$ 5 ug/kg $Ah^{d/d}$	5 ug/kg $Ah^{b/b}$ 10 ug/kg $Ah^{d/d}$	Kerkvliet et al., 1990a
B6C3F1 mice	single i.p injection of 0, 0.1, 1.0, 10 ug/kg	Spleen cell IgM, IgG, TNP-LPS, and TNP-Ficoll AFC response/10^6 recovered cells	0 ug/kg 0.1 1.0 10	0 IgM AFC -3% -57%* -77%*	0 IgG AFC -14% -22% -42%*	0 TNP-Ficoll -28% -66%* -91%*	0 TNP-LPS N/A N/A +6%	IgM 0.1 IgG 0.1 TNP-Ficoll 0.1	IgM, IgG, and TNP-Ficoll 1.0	House, et al., 1990
Male C57BL/6 male mice	weekly i.p injectionX 4 wks week 5 injected with 1-2 X 10, 5 days later inj. $2x10^8$ SRBC	measured footpad 24 hrs after challenge	0 0.4 ug/kg TCDD 4 ug/kg TCDD 40 ug/kg TCDD	0 Response footpad swelling -3% 34% -38%						Clark, et al., 1981
Female B6C3F1 mice	14 daily doses p.o. TCDD	1. Serum complement activity CH50 representing the amount of serum necessary to lyse 50% of the target cells 2 Serum level of complement component C3	0 Total dose ug/kg/day 0 01 0 05 0.1 0.5 1.0 2 0	0 CH50 %decrease 41%* 31%* 38%* 54%* 65%* 81%*		0 serum C3 %decrease 3% 6% 2% 8%* 19%* 26%*		CH50= N/A C3 = 0 1	CH50=0 01 C3 = 0.5 ug/kg/day	White, 1986

Table VI
Dose - Response Effects of TCDD on PFC response in C57BL/2N and B6C3F1 Mice

Species	Dosing Regimen	Effect	Dose	% Response		NOAEL	LOAEL	Reference
C57BL/6N mice male	Single i.p injection with TCDD followed 5 days by a single i.p. injection of 4 X 10^8 SRBC	% decrease in spleenic plaque forming cells/10^6 spleen and per viable cells 4 days after SRBC treatment	0 ug/kg 0.322 0.644 1.19 3.09	0% 6.3% 43.6% 84.8% 91.6%	PFCs/10^6 spleen 777 731 438 118 65	N/A	N/A	Davis and Safe, 1988
C57BL/6J Female	single oral dose TCDD 2 days prior to single i.p. injection 2.5 X 10^{-8} SRBC	% decrease in spleenic plaque forming cells/10^6 spleen cells	0 ug/kg 0.2 1 2 5	0 % 30.4 % 52.6 % 67.4 % 78.3 %				Kerkvliet and Brauner, 1990
Male BALB/cByJ mice	single oral dose TCDD administered orally 2 days prior to injection with SRBC	% decrease PFC/10^6 spleen cells	0 ug/kg 0.25 1.0 4.0 16.0	0 % 6.6 % 18.2% 79.6% 98.6%		1.0 ug/kg	4.0 ug/kg (all doses were decrease)	Silkworth et al., 1989
DBA/2J male mice	single oral dose TCDD administered orally 2 days prior to injection with SRBC	% decrease PFC/10^6 spleen cells	0 ug/kg 0.25 1.0 4.0 16.0	0 % 23.5% 32.6% 57.1% 87.9 %			at all doses significant decrease observed	Silkworth et al., 1989
B6C3F1 Mice female		% decrease in spleenic plaque forming cells/ 10^6spleen and per viable cells 4 days after SRBC treatment	0 ug/kg 0.005 0.010 0.025 0.050 0.1 0.5 1.0 2.5	0% -7.8% -18.6% 2.3% 13.1% 55.7% 62.7% 84.5% 92.5%	939 1012 1114 917 816* 416* 350* 146* 70*	0.025 ug/kg	0.05 ug/kg	Narasimhan et al., 1993

Complement is another important factor in the immune response. White et al. (1986) investigated the effect of TCDD on complement levels and activity in female B6C3F1 mice administrated 0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.0 ug TCDD/kg total dose administered over 14 day period. As a measure of complement activity White estimated CH_{50} which is the amount of treated mouse serum required to lyse 50% of the cells. There was a significant decrease in (CH_{50}) activity at all TCDD doses tested (Table V). A significantly reduced level of the C3 complement component was found at 0.5 ug TCDD/kg. However, the C3 component was the only component measured. It is concluded that several components may be affected, hence the decrease in CH_{50} activity is a more sensitive indicator of toxicity to the complement system that individual components.

In summary, immune system may be profoundly affected by TCDD administration, and may prove to be the most sensitive indicator of TCDD toxicity. A broad spectrum of immunotoxic effects have been reported after TCDD administration in adult animals. Significant among these are the increases in mortality in TCDD treated mice challenged with influenza (House et al. 1990) and the decrease in complement (CH50) activity in which the LOAELs were both less that 0.01 ug TCDD/kg. In addition the PFC assay has been found to be an extremely sensitive indicators of toxicity (Narasimhan et al. (1993) found LOAEL = 0.05 ug TCDD/kg). Without studies comparing similar endpoints across species the implications of this toxicity is difficult to assess. It has been suggested that the susceptibility of the developing immune system is even greater than that of adult animals. This and other effects on the developing fetus are very sensitive to TCDD administration.

D. Teratogenic/Developmental Toxicity

The effects of TCDD on proliferative cellular events make it a particular concern in the developing fetus. Although only 0.0005% of maternally administered TCDD reaches affected tissues in the fetus of the C57BL mouse (Abbott and Birnbaum 1989), many effects including thymic atrophy, cleft palate, and hydronephrosis are observed (Couture et al. 1990). The responses to maternally administered TCDD are dose dependent, and based on studies of sensitive and resistant strains of mice, appear to segregate with the Ah receptor locus (Poland and Glover 1980; Silkworth et al. 1989).

D.1. Cleft Palate

Couture et al. (1990) summarized 23 studies which described the teratogenic and developmental toxicity of TCDD. Of the 14 studies summarizing toxicity in mice, 13 listed cleft palate and 8 listed hydronephrosis or renal congestion as toxic endpoints. None of the remaining 9 studies, performed in other species, list cleft palate as an endpoint, and only one study in hamsters described hydronephrosis. All species have not been studied, yet it is believed that cleft palate is unique to mice. Although hydronephrosis is believed to occur via a mechanism related to cleft palate it has been reported to occur in other species including rat and hamster. Various protocols have been used to evaluate cleft palate and hydronephrosis, the two most common fetal abnormalities, and generally the response is dose dependent.

Mice, because of their unique sensitivity to cleft palate, have been the focus of the research on the developmental and teratogenic effects of TCDD. Coutney and Moore(1971) observed an increase in the incidence of cleft palate and kidney anomalies in three strains of mice DBA/2J, C57BL/6J and CD-1 after dams were administered 3 ug TCDD/kg/day s.c for 10

days (GD 6-15). In the CD-1 mice a dose dependent increase in cleft palate incidence and kidney anomalies was observed in offspring administered 1 and 3 ug TCDD/kg/day. Courtney and Moore (1971) also observed that CD rats administered 0.5 ug TCDD/kg/day did not have offspring with an increase in cleft palate. The authors concluded that the increase in cleft palate was specific to the mouse, however, there was an increased incidence of kidney anomalies (not characterized) in these rats. In addition, no significant maternal toxicity (characterized as decreased maternal weight gain or increase relative liver weight) was observed in these CD rat or CD-1 mice. The effects of TCDD administered orally 0, 0.001, 0.01, 0.1, 1.0 and 3.0 ug TCDD/kg/day on gestation days (GD) 6-15 on fetal development in Carworth CF-1 mice was investigated by Smith et al.(1976) (Table V). At the 1.0 and 3.0 ug TCDD/kg/day dose a statistically significant increase in cleft palate was observed. At the highest dose an increase in dilated renal pelvis was observed. Since there was a response at only two doses it is difficult to conclude if there is a dose-response relationship using this protocol. The NOAEL for developmental /teratologic effects was reported as 0.1 ug TCDD/kg/day (1.0 ug/kg total dose), and LOAEL was 1.0 ug TCDD/kg/day (10 ug/kg total dose), doses comparable to that of Courtney an Moore (1971).

Courtney (1976) also investigated the teratogenic effects of TCDD using oral and subcutaneous routes of administration . Pregnant CD-1 mice were administered 0, 25, 50, 100, and 200 ug TCDD/kg/day using both routes of administration, and additional dose of 400 ug TCDD/kg P.O. for 10 days (GD 6-15). Maternal toxicity, evident in all dose groups, was characterized by a decrease in maternal body weight gain, an increase in relative liver weight, and in some cases marked edema. Parameters used to assess fetotoxicity were number of live fetuses/litter, number of abnormal fetus/litter, percent cleft palate, percent kidney anomalies (characterized as

hydronephrotic kidneys), and percent pups with a club foot. A dose-dependent increase in percent cleft palate and percent hydronephrosis was detected in all groups administered TCDD orally, except at the highest dose where it was observed that the percent kidney anomalies decreased probably because of the extent of maternal toxicity at this dose. (Table VII). The mice administered TCDD s.c. had a response (increase cleft palate) close to maximal at the lowest dose tested, although the same dose administered orally resulted in a minimal but detectable response. Additionally, the comparison of 2,3,7,8-TCDD to 1,2,3,4 TCDD and OCDD shows 2,3,7,8 TCDD to be the most potent fetotoxin of these congeners tested. The doses in this study were extremely high and do not reflect the sensitivity of cleft palate and hydronephrosis as toxic endpoints (Courtney 1976). The CD-1, CF-1, C57BL/6J and the DBA mice clearly have different sensitivity to TCDD induced cleft palate. An incidence of 19% cleft palate was observed in CD-1 mice administered 25 ug TCDD/kg/day orally GD 7-15, while only 1.0 ug TCDD/kg/day in the CF-1 mouse (the most sensitive of the species tested) resulted in 21% cleft palate in offspring (Smith et al. 1976). C57BL/6J administered 4 ug TCDD/kg/day and DBA administered 8 ug TCDD/kg/day had 47% and 27% cleft palate incidence respectively (Table VII) (Silkworth et al. 1989a). After the initial reports that TCDD was teratogenic in mice, the focus of research was to characterize these fetal anomalies and on finding a sensitive period during gestation when these effects occurred. Unfortunately, because of the study designs, fetal tissue dose and distribution was not obtained, therefore, quantificative assessment of dose response relationships can not be done.

To determine if there is a sensitive period that cleft palate or hydronephrosis occurred, Birnbaum et al. (1989) and Weber et al. (1985) administered TCDD to mice on GD 10 or 12. In

the offspring of pregnant C57BL/6N mice administered a single oral dose on GD 10 of 0, 12, 17, and 22 ug TCDD/kg, a significant and dose-dependent increase in percent of fetuses with cleft palate was observed. Maternal toxicity characterized as decreased weight gain and increased relative liver weight was significant at the 17 ug TCDD/kg dosage level.(Weber, et al., 1985). However, kidney damage which was significant at all levels, was not clearly dose dependent because of a near maximal response at the lowest dose. These doses are equivalent to the doses administered for 10 days to the CF-1 (Smith, et al. 1976) mice which produced cleft palate and hydronephrosis. Similar doses were also used by Birnbaum et al. (1989) to assess the teratogenic effects of TCDD in C57BL/6N mice. Pregnant mice were administered a single oral dose of 0, 6, 12, 15, or 18 ug TCDD/kg on GD 10, or doses of 0, 6, 9, 12, 15 ug TCDD/kg on GD 12 (Table VII). On GD 10 and GD 12 maternal toxicity characterized as increase relative liver weight was observed at all doses. A statistically significant dose-related increase in cleft palate was observed at 12 ug TCDD/kg on GD 10 and at 9 ug TCDD/kg on GD 12. Maximal cleft palate induction was observed in C57BL/6N mice administered TCDD on gestation day 12. The observed incidence was about 60% greater than the incidence observed at the same dose (15 ug TCDD/kg) on GD 10. Couture et al. (1990a) reported, in an experiment using single dose administration in C57Bl/6J mice, that on GD 10 or 12 a LOAEL of 12 ug TCDD/kg for cleft palate, and a LOAEL of 3 ug TCDD/kg on GD 6, 8, 10, 12 or 14 for hydronephrosis.

To determine when palate formation was most sensitive to TCDD, Pratt et al.(1984) administered a single s.c. dose of 100 ug TCDD/kg to C57BL/6J mice on days 3-13 of gestation. Greater than 95% of the fetuses from dams administered TCDD on days 8,9,and 10 had cleft palate.

The maximum response was observed on day 10. Histological examination of palate formation in controls revealed significant cell death of medial epithelial cells resulting in palate closure. In the TCDD treated mice this cell death was not observed. This apoptosis (programmed cell death) is apparently inhibited by an Ah receptor mediated mechanism (Pratt et al. 1984). The role of the Ah receptor in the teratogenic effects of TCDD after repeat dose administration was investigated by Silkworth et al. (1989a). By comparing C57BL/6J and DBA/2J mice Silkworth determined that the incidence of cleft palate segregated with the Ah receptor. Pregnant C57BL/6J mice were administered 0, 0.5, 1.0, 2.0, and 4.0 ug TCDD/kg/day during gestation day 6-15 (total dose of 0, 5, 10, 20, and 40 ug TCDD/kg). DBA/2J were administered up to 8.0 ug TCDD/kg/day.(Table V) The most sensitive indicators of maternal effects in C57BL/6J mice was an increase in relative liver weight and a decrease in relative thymus weight seen at all doses. A significant increase in relative liver weight and a decrease in the relative thymus weight was seen in the DBA mice at the 2.0 ug TCDD/kg/day and 8.0 ug TCDD/kg/day dose level, respectively, indicating a 4-8 fold decrease in TCDD sensitivity in the DBA /2J mice consistent with other studies. In both strains of mice there was a statistically significant increase in percent cleft palate/dam at the highest doses 4 and 8 ug TCDD/kg/day. These doses were 4-8 times higher than the LOAEL for maternal effects. This study indicated that the fetal abnormality (cleft palate) seen in DBA and C57BL/6J mice was a less sensitive indicator of TCDD's effects than the measures of maternal effects, but that percent of offspring with hydronephrosis was more sensitive being significantly increased in both strains at 0.5 ug TCDD/kg/day. This is not true with all mice strains. In the CF-1 mice studied by Smith et al. (1976) using a similar protocol, the teratogenic effects (cleft palate) of TCDD proved to be

a more sensitive indicator than maternal effects characterized as increase relative liver weight. Most importantly this comparison of C57BL/6J and DBA/2J points out that in these two mouse strains a statistically significant increase in hydronephrosis is observed at the same dose suggesting this may be an equitoxic measure of maternal dosing of TCDD, however the magnitude of this response is not equivalent at the LOAEL, 5 ug/kg.

Identifying the window in which maximal cleft palate occurred enabled Abbott and Birnbaum (1989) to investigate the mechanism by which TCDD disrupted medial epithelial cell terminal differentiation. Levels of EGF receptor were found to be a critical difference in the development of the embryonic palate in the presence of TCDD. Using the C57BL/6N, Abbott and Birnbaum, administered a single oral dose of TCDD on GD 10 or GD 12. Four days post dosing measurements of thymidine incorporation, concentration of EGF receptor, and degree of palate closure was shown to be markedly increased. It was concluded that the characteristic TCDD mediated cleft-palate formation is due to a defect in the fusion of the palatial shelves. Although the shelves meet, the medial cells which usually die during palate closure continue to proliferate under the influence of TCDD. The mechanism by which this occurs is shown to be related to the increase expression of the EGF receptor in the medial epithelium on GD 10 and 12, which coincides with the sensitive time for cleft palate formation (Abbott et al. 1989a).

The incidence of cleft palate in mice correlates well with their sensitivity to Ah receptor mediated toxicity. Poland and Glover (1980) administered a single s.c. dose of 30 ug TCDD/kg to pregnant dams of 10 different mice strains. Four of the five TCDD sensitive strains had a 54-95% incidence of cleft palate, whereas all the strains with a low sensitivity to Ah receptor mediated

toxic effects had a < 4% incidence of cleft palate. Poland and Glover also investigated the strain differences using C57BL/6J, DBA/2J and their hybrid cross B6D2F1/J mice. Mice were administered a single s.c. dose of 0, 3, 10, or 30 ugTCDD/kg on GD 10. A dose-dependent increase in cleft palate/live fetus was seen in the C57BL/2J mice (Table VII). In the other two strains cleft palate was only seen at the highest dose indicating a segregation with the Ah receptor.

Dencker and Pratt (1981) investigated the role the tissue concentration of the Ah receptor plays in cleft palate formation using two strains of mice C57BL/6J, and AKR/J (a TCDD resistant strain). The results of this comparison indicate that the concentration of Ah receptor is greater in the maxillary process (the region containing palate shelves) of the C57BL/6J mice than in the AKR/J mice. In addition,the embryonic C57BL mice maxillary process contained a higher concentration of Ah receptor (measured on GD 13) than the embryonic brain, liver, limb buds or skin. The Ah receptor without doubt has some role in cleft palate formation probably involving the altered transcription of a protein involved in palate epithelial cell division or apoptosis (Dencker and Pratt 1981).

Experiments using cultured embryonic palate tissue have enabled researchers to observe palate development more closely and to compare very sensitive endpoints in mouse, rat and human tissue. Abbott and Birnbaum (1989) established this methodology using C57BL/6N mice palate shelves. An extensive database of in vivo data has made this comparison possible. GD 12 mice palate shelves were exposed to 0, 10^{-13}, 10^{-12}, 10^{-11}, 5 X 10^{-11}, 7.5 X 10^{-11}, 10^{-10}, and 10^{-9} M TCDD (Table VIII for dose equivalents). A dose-response was observed in percent of palates not undergoing apoptosis (an endpoint that indicates the medial epithelial cells continue to grow resulting in cleft palate formation). The response increases until reaching the cytotoxic

concentration of 7.5 X 10^{-11}M. The EGF receptor, measured by immunochemistry, increased in medial epithelial cells at 10^{-11}, 5 X 10^{-11}, 10^{-10}, and 10^{-9} M (only doses reported). An increase in cell proliferation (thymidine incorporation) was also found to increase significantly. Table VIII points out the relationship between in vitro culture concentrations and administered dose. These calculations are based on the observation that 0.0003% of a 30 ug TCDD/kg dose administered to a pregnant C57Bl/6N mouse is distributed to the palate tissue (Abbott and Birnbaum 1989). The concentrations that produce cleft palate in vitro correlate well with the doses that are found in vivo to produce cleft palate. For example, Birnbaum et al. (1989) found an increase cleft palate after a single dose administration of 12 ug TCDD/kg. Abbott and Birnbaum (1989) found a significant increase in EGF receptor levels in medial epithelial cells at all dose above 10^{-11} (equivalent to 20 ug TCDD/kg). Decreased program cell death was observed at dose equivalents of 0.2 ug TCDD/kg to 200 ug TCDD/kg.

Rats are not as sensitive to cleft palate formation as are mice. This appears to be because TCDD is fetotoxic and maternally toxic. Therefore, fetal death may occur at lower doses than cleft palate formation. The pups of Holtzman rats administered 0, 1.5, 3, 6, and 18 ug TCDD/kg on GD 10 developed cleft palate only at the 18 ugTCDD/kg dose. A dose-dependent increase in percent fetal mortality and decrease in fetal thymus weight was observed in these rats. Although cleft palate was not observed at the low doses, the percent of fetuses per litter affected by intestinal hemorrhage was significantly increased in a dose-dependent manner at all doses (Olson 1989, 1990). Other endpoints were also found in other animal species, guinea pigs were treated orally with 0, 0.15 and 1.5 ug TCDD/kg on GD 9 and a significant increase in percent fetal mortality occurred at the 1.5 ug TCDD/kg dose (Olson 1990). The developmental toxicity of Golden Syrian

Hamsters was investigated (Olson 1990) after administration of 0. 1.5, 3, 6, or 18 ug TCDD/kg on GD 9. A significant dose related increase in relative liver weight was observed in dams at all doses. In addition, a significant dose-dependent increase in neutrophilic metamyelocytes, neutrophilic band cells, total liver P450 activity, and EROD activity was observed at all doses. A dose-dependent decrease in liver estradiol 2-hydroxylase activity was observed in the dams, which was significant at the 3 ug TCDD/kg dose level. Fetal liver EROD and total P450 also increased at all doses, but statistical significance was not determined due to small sample size. Fetal thymus area decrease and kidney congestion was observed at all dose levels. A dose-dependent increase in the incidence of hydronephrosis was observed and was statistically significant at the 3.0 ug TCDD/kg (maternally administered) dose level (Olson et al. 1990). The hamster is the least sensitive species tested to the acute toxic effects of TCDD (see Table II), but it is extremely sensitive to the developmental abnormalities observed. Kidney congestion and hydronephrosis are observed after a single dose of 1.5 ug TCDD/kg which is equivalent to the toxicity seen in the CF-1 mouse (Table V). This observation raises questions regarding the ability of acute toxicity to predict tissue specific sensitivity.

The in vitro palate culture provides a method to examine palate development in rats without the associated maternal or fetotoxicity of TCDD. Abbott and Birnbaum (1990) culturing GD 14 palates of F344 rats with TCDD, found a dose-dependent increase in the number of palates in which the medial peridermal cells did not go through programmed cell death (Table VIII). Since data on distribution of TCDD to the rat fetal palate has apparently not been reported it is not possible to determine the comparable administered dose. It is valuable to compare the tissue dose response in several species. The mouse is about 100-1000.

Table VII TERATOGENIC EFFECTS: DOSE RESPONSE

Species	Dosing Regimen	Effect	Dose		Response		NOAEL	LOAEL	Reference
Carworth CF-1 mice	oral dose administered daily GD 6-15	percent liters with cleft palate	0 ug/kg/day 0.001 0 01 0.1 1 3	0 ug/kg tot. dose 0.01 0 1 1.0 10 30	0 % palate 5% 0 % 6 % 21 %* 71 %*	0% kidney 0% 0% 0% 5% 28%*	0.1 ug/kg/day	1 0 ug/kg/day 10 ug/kg	Smith et al., 1976
CD-1 mice	1. daily oral dose administration 2. daily s.c. dose administration GD 7-16	% cleft palate /total fetus	0 ug/kg/day 25 50 100 200 400	0 ug/kg tot. dose 250 500 1000 2000 4000	0 1.oral 3% 19% 66% 100% 100%	0 2 s.c. 82% 79% 85% 100%	N/A	N/A	Courtney, 1976
CD-1 mice	1. daily oral dose administration 2. daily s.c. dose administration GD 7-16	% kidney anomalies/total fetus	0 ug/kg/day 25 50 100 200 400	0 ug/kg tot. dose 250 500 1000 2000 4000	0 1.oral 34% 72% 71% 100% 50%	0 2. s.c. 53% 58% 95% 38%	N/A	N/A	Courtney, 1976
C57BL/6J mice	single oral dose administered on GD 10	1. percent liters with cleft palate 2. percent fetuses with cleft palate	0 ug/kg 12 ug/kg 17 ug/kg 22 ug/kg		1. 0% 40% 82% 100%	2. 0% 8.7%* 43.9%* 77.6%*		12 ug/kg	Weber et al., 1985
C57BL/6J mice	daily administration GD 6-15	1. % cleft palate per dam 2. % hydronephrosis	0 ug/kg/day 0.5 1.0 2.0 4.0	0 ug/kg Total dose 5.0 10 20 40	1. 0% 0% 2.23% 2.33% 47.23%*	2. 0% 39.92%* 72.79%* 74.23%* 91.83%*	1. 20 ug/kg 2. 5 ug/kg	4 ug/kg/day	Silkworth et al., 1989a
DBA/2J mice	daily administration GD 6-15	1.% cleft palate per dam 2.% hydronephrosis	0 ug/kg/day 0.5 2.0 4.0 8.0	0 ug/kg Total dose 5 20 40 80	1. 0% 0% 3.51% 3.47% 27.24%*	2. 0% 4.95* 13.16* 36.89* 60.47*	1. 40 ug/kg 2. 5 ug/kg	8 ug/kg	Silkworth et al., 1989a
C57BL/6N mice	single oral administration on GD 6,8,10,12	1. % fetuses/litter with hydronephrosis 2. % fetuses/litter with cleft palate	Dose 0 ug/kg 3 6 9 12 24	1. GD 6 25.7% 96.2% 100.0% 100.0% 100.0% 100.0%	2. GD 6 0.0% 0.0% 1.0% 0.0% 3.5% 40.3%*	1. GD 8 2. GD 8 27.0 0.3 90.0 0.0 - - - - 100.0* 9.7* 100.0* 93.4*	1. GD 10 2. GD 10 18.6 0.0 89.2 0.0 - - - - 100.0* 53.9* 100.0* 98.8*	1. GD 12 2. 6.4% 0.0 97.9%* 1.1 - - - - 100.0%* 76.0%* 100.0%* 100%*		Couture et al., 1990a
1. C57BL/6J 2. B6D2F1/J 3. DBA/2J mice	single s.c. administration GD 10	% cleft palate/live fetus	0 ug/kg 3 ug/kg 10 ug/kg 30 ug/kg		1. 0% 0 3 54	2. 0% 0 0 13	3. 0% 0 0 2			Poland and Glover, 1980
Golden Syrian Hamsters	single oral dose GD 10	1. % kidney congestion 2. % hydronephrosis 3. % fetal mortality	0 ug/kg 1.5 3.0 6 18	1. 0 9.7* 28.5* 83.6* 91.1*	2. 1.6% 11.2% 15.3%* 34.5* 0	3. 7% 10.8% 7.7% 8.7% 58.2%			Olson, 1990
C57BL/6N Mice	single oral dose administered to dam on GD 10 or GD 12	increase cleft palate incidence	0 ug/kg GD 10 6 12 15 18	0 ug/kg GD 12 6 9 12 15	0 GD 10 incidence 0 20.86* 49.30* 70.12*	0 GD 12 incidence 1.43 19.91* 51.45* 77.91*	GD 10 = 6 ug/kg GD 12 = 6 ug/kg	GD 10=12 ug/kg GD 12=9 ug/kg	Birnbaum, et al., 1989

times more sensitive when examining the endpoint of programmed cell death. Hence, it may be concluded that tissue distribution of TCDD is not the rate limiting factor for species sensitivity. Human effects of TCDD relating to pregnancy are difficult to determine, primarily because of the confounding effects of multiple potential etiologic agents, a relatively high background of birth defects, and a lack of robust exponential data. The cultured human palate allows direct comparisons between similar endpoints that have been evaluated in the rat and mouse species. Abbott and Bimbaum (1991) cultured human palatine tissues GD 52, 53, and 54 for 3-4 days. Evaluation of palate growth and development revealed that 100% of the palate medial epithelial cells continued to proliferate and scheduled programmed cell death did not occur at 10^{-8} and 10^{-9} M TCDD concentrations. Since only three concentrations were reported, a dose-response relationship is difficult to evaluate although there was an increased response observed between the 10^{-11} and the 10^{-8} M concentrations. Direct comparison between the human and mouse palate demonstrates that the concentrations at which 25% or 100% of palates were affected (programmed cell death did not occur) were 500 times more sensitive in the mouse than in the human palate (See Table VIII). These in vitro observations found that the human palate is sensitive to cleft palate formation through the same mechanism seen in mice; changes in growth factors (ie; EGF, and TGF's) that are involved in the mechanism of altering programmed cell death in the medial epithelial cells of the palate. The effects of TCDD on regulation of these growth factors causes epithelial cells to continue to proliferate resulting in palatine shelves that do not fuse normally.

Table VIII: Comparison of Effects of TCDD on In Vitro Palate Cultures				
Species	Gestation Days	Concentrations	Response	Reference
C57BL/6N mice	GD 12	Control 1 X 10^{-13}M 1 X 10^{-12}M 5 X 10^{-12}M 1 X 10-^{11} M 5 X 10-^{11}M 7 5 X 10-^{11}M 1 X 10-^{10}M	6 % without Programmed Cell Death 25 % without PCD 38 % without PCD 66 % without PCD 66 % without PCD 100 % without PCD 83 % without PCD 50 % without PCD	Abbott et al , 1989a
F344 rats	GD 14-15	Control 5 X 10^{-11}M 1 X 10^{-10}M 1 X 10^{-9}M 1 X 10^{-8}M	5 % without Peridermal Cell Death 0 % without Peridermal Cell Death 20 % without Peridermal Cell Death 36 % without Peridermal Cell Death* 60 % without Peridermal Cell Death*	Abbott and Birnbaum, 1990
Human Palate Cultures	GD 52, 53 and 54	Control 5 X 10^{-11} M 1 X 10^{-8} M 1 X 10^{-7} M	0 % without Peridermal Cell Death 25 % without Peridermal Cell Death 100 % without Peridermal Cell Death 100 % without Peridermal Cell Death	Abbott and Birnbaum, 1991

Alteration of EGF has been reported in other cell systems. Hudson et al. (1985) found a concentration-dependent decrease in EGF receptor binding after treatment with TCDD in human keratinocyte cell line. These binding assays demonstrated two binding components for EGF; high and low affinity. Cells treated with TCDD have a loss of the high affinity binding component. TCDD does not directly bind to the EGF receptor and the loss of binding the high affinity receptor may be due to an alteration in other binding factors. The decreased binding affinity in human keratinocytes is associated with a decrease in DNA synthesis. In contrast, palate epithelial cells treated with TCDD in culture were found to have an increase in EGF receptor level and a concomitant increase in cell proliferation. The responses in the human keratinocyte and human palatine cells appears to be very different. This may indicate an intrinsic difference in cellular response to cell specific growth factors and subsequent differences in regulation of cell growth.

D.2 Hydronephrosis

Hydronephrosis is the second well characterized fetal anomaly observed commonly in TCDD treated mice. In contrast to cleft palate which is species and tissue specific for TCDD treated mice, kidney toxicity occurs after pre and postnatal treatment, and occurs in several species. The kidney lesions have been characterized as an increase cell proliferation of the epithelial cells of the uteric lumen. Cell proliferation causes obstruction/constriction of the ureter resulting in decreased urine outflow and "true hydronephrosis" (Abbott et al. 1987). Uteteric epithelial hyperplasia has been observed in mice administered TCDD both pre and postnatally, in adult guinea pigs (McConnell et al. 1978a) in Rhesus monkeys (Gupta et al. 1973) and reported as kidney anomalies,but not further characterized in CD-rats (Courtney and Moore 1971).

TCDD administered to pregnant C57BL/6 mice (GD 10-13) at 1.0, 3.0 and 10.0 ug TCDD/kg/day (total dose 4, 12, 40 ug/kg) resulted in a dose dependent increase in the incidence of early stages of hydronephrosis and "renal papilla which are markedly reduced in size, or non-existent, in a few cases resulting in an enlarged renal pelvis." These lesions were found predominantly in the right kidney. The lesions were also found in pups exposed postnatally and were qualitatively similar to lesions found in fetuses exposed in utero (Moore et al. 1973). Fetal hydronephrosis is a very sensitive indicator of TCDD toxicity with an incidence of 89-98% observed after administration of a single dose of 3 ug TCDD/kg during GD 6, 8,10, or 12 (Couture et al. 1990a).

In mice hydronephrosis appears to be the most sensitive indicator of fetotoxicity. Silkworth et al. (1989a) demonstrated this by administering C57BL/6J and DBA/2J mice 0. 0.5, 1, 2, and 0, 0.5, 2,4,8 ug TCDD/kg/day respectively during GD 6-15. A statistically significant increase in hydronephrosis was observed at the lowest dose tested 0.5 ug TCDD/kg/day (total dose 5 ug

TCDD/kg) in both strains of mice with no NOAEL observed for this endpoint. In contrast cleft palate incidence was observed to be statistically significant (Table V) at the 4 ug TCDD/kg/day (40 ug/kg total dose) in C57Bl/6J mice and at 8 ug TCDD/kg/day (80 ug/kg total dose) in the DBA/2J mice. The sensitivity of these endpoints may be related to the window of sensitivity in fetal development that these anomalies can occur. Studies determining the mechanism of each of these lesions have helped to understand these differences.

Couture et al. (1990a) administered a single oral dose to C57BL/6N mice on GD 6, 8, 10, 12 or 14 to investigate the point in gestation that TCDD produced maximal toxicity. Unfortunately, all administered doses (3, 6, 9, 12, 24 ug TCDD/kg produced a statistically significant increase in the incidence of hydronephrosis from GD 6-14 so a sensitive time period was not determined (Table VII). The incidence appeared to decrease after day 14. Couture-Haws et al. (1991, 1991a) examined sensitivity to TCDD administered postnatally in dams administered 0, 6, 9, 12 ug TCDD/kg on postnatal day 1, 4, 8 and 14. All doses significantly increased the incidence of hydronephrosis on day 1 and 4. No increase was seen on PND 8 or 14. No maternal toxicity was observed at any dose. These results indicate that the neonatal kidney is affected by the level of TCDD available in milk.

The mechanism is similar to that for cleft palate since altered regulation of cell growth and death are responsible for cleft palate formation in the mouse. This is mediated through a sustained level of EGF production not found in control animals. TCDD induced hydronephrosis in the mouse is characterized as an increase in uteric epithelial cells causing closure of the ureter. The continued proliferation of these epithelial cells as measured by thymidine incorporation is found to correlate with a sustained level of EGF receptors (Abbott and Birnbaum 1990a).

Cultured ureteric epithelial cells derived from GD 10 or 12 mouse fetuses had an incidence of 91-100% hyperplasia after exposure to 1 x 10^{-10} M (32 pg/ml) TCDD, a 4 fold increase over control. In addition, intense EGF receptor staining (immunohistochemistry) and increase thymidine uptake(indicating cell growth and proliferation) was observed in most of these sections (Abbott and Birnbaum 1990a). Based on the LOAEL of hydronephrosis seen in C57BL/6N mice in vivo (3 ug TCDD/kg) the tissue dose seen in the ureter of these mice is equivalent to 0.3 pg (Abbott and Birnbaum 1990a)

When comparing TCDD toxic endpoints, the effects on fetal development do not appear to be the most sensitive in all of the species studied. However, without information on tissue distribution in all species this conclusion is somewhat limited.

Hydronephrosis is a more sensitive fetotoxic endpoint in the mouse and in the hamster occurring at doses that are not overtly toxic to dams. Cleft palate on the other hand appears to be a TCDD-related teratogenic effect unique to the mouse, but analysis of the mechanism reveals that the interference with programmed cell death by TCDD is not unique to the mouse. Cleft palate is found at doses where maternal effects are observed (characterized as decrease weight gain or increase relative liver weight) in C57BL and DBA mice, but cleft palate is <u>not</u> accompanied by maternal effects in the CD-1 or CF-1 mice, although these strains have not been thoroughly examined.

D.3 Other

Giavani et al. (1982, 1982a, 1982b) attempted to characterize cleft palate and kidney abnormalities in rabbits and rats. The most frequently observed lesion among litters of pregnant rats administered 0. 0.125, 0.5 and 2 ug TCDD/kg/day during GD 1-3 was cystic kidneys. This

was not statistically significant and was observed at doses that were fetotoxic and maternally toxic, but it does demonstrate that kidney development is a target of TCDD toxicity when administered early in gestation (Giavini et al. 1982a). Pregnant rabbits administered 0. 0.1, 0.25 0.5 and 1 ug TCDD/kg/day GD 6-15, TCDD had a significant increase in post-implantation losses reaching 100% at the highest dose. At maternally toxic and fetotoxic doses, a small increase in kidney abnormalities characterized as hydronephrosis and double kidney formation was observed (Giavini et al. 1982b). These observations indicate that the development of kidney anomalies, although found, are not the most sensitive indicator of fetotoxicity in the rabbit or the rat.

Giavinni et al. 1982 investigated the effects of TCDD on the implantation characterized as mean corpus lutea, mean number of implantation, and percent preimplantation losses in Sprague-Dawley rats. Dams were administered 0, 0.125, 0.5, and 2.0 ug TCDD/kg/day for 3 days GD 1-3 (total doses 0, 0.375, 1.5, and 6 ug/kg). Fetotoxicity measured as fetal weight on GD 21, and maternal toxicity measured as weight gain was also evaluated. Maternal toxicity was observed at the highest dose only, and fetotoxicity was observed in the 0.5 and the 2 ug TCDD/kg/day dose group. The results indicate that no significant relationship between TCDD at doses up to 2 ug TCDD/kg/day and pre or post implantation losses, mean corpus lutea, or number or implantation sites. Fetoxicity was found to be related to maternal toxicity (Giavini et al. 1982) as suggested by Khera (1987), who postulated that maternal toxicity may be responsible for the fetotoxicity found after administration of certain chemicals. In Sprague-Dawley rat (Sparschu et al. 1971) administered 0.03, 0.125, 0.5, 2.0 and 8.0 ug TCDD/kg GD 6-15 the most sensitive indicator of fetotoxicity was decrease fetal weight and gastrointestinal hemorrhage LOAEL = 0.5 ug TCDD/kg/day. This occurred at a dose that was not maternally toxic although decrease fetal weight

may be associated with undetected maternal toxicity. Gastrointestinal hemorrhage was also the most sensitive indicator of fetal toxicity observed in Holtzman rats by Olson (1990). Dams administered 0. 1.5, 3, 6, and 15 ug TCDD/kg single p.o. dose on GD 10 had a statistically significant increase in percent fetuses per litter affected at all doses administered.

Evaluation and comparison of reproductive endpoints are complicated by the predominance of species specific effects and the unique problems of fetal/tissue distribution. Understanding the underlying biochemical mechanisms responsible for adverse reproductive effects is essential to proceeding with comparative analysis. For cleft palate these mechanisms have been thoroughly investigated and any risk assessment will be enhanced by incorporating this information.

E. Hormone Effects

In the classic TCDD carcinogenicity study (Kociba et al. 1978) it was observed that female rats had a dose-dependent increase in liver cancer and a dose-dependent decrease in the incidence of spontaneous tumors in the uterus, breast and pituitary. These observations, coupled with reports suggesting that the mechanism of TCDD toxicity was mediated through a nuclear hormone-like receptor mechanism (Poland and Knutsen 1982) led researchers to investigate the role of hormones in modulating TCDD toxicity. Early work focused on thyroid hormone and the role of the thyroid in the toxicity of TCDD (McKinney et al. 1985; Lucier, this document) The possible role of estrogens and estrogen receptor in the toxicity of TCDD was recognized by several research teams (Gallo and Umbreit 1988). The estrogen receptor and the Ah receptor are both ligand-specific and response element specific (Denison 1991, Green and Chambon, 1991). Experimental evidence (Poland and Glover, 1980; Romkes and Safe, 1988) indicates that TCDD does not bind the

estrogen receptor(ER) nor does estradiol(E2) bind to the Ah receptor. However, there may be direct competition for factors that modulate ligand-receptor binding, translocation, and response element binding ie., heat-shock protein 90 (Perdew 1988, Green and Chambon, 1991). It may be the effect of TCDD on the concentration of uterine and hepatic ER that results in modification of estrogens sensitive tissues. In addition, subsequent alteration in estradiol levels from induction of CYPIA1 and CypIA2 may induce organ specific changes (DeVito et al. 1992a). Altered estradiol levels have also been implicated in a reduced immune response (Luster et al. 1985), and both an increase and decrease in tissue specific cancers. The relationship between TCDD, estradiol and cancer detailed in the Carcinogenicity Chapter of this document(Lucier) is still not fully understood.

The dose response relationships between TCDD and altered Estrogen Receptor(ER), and/or changes in serum estradiol can result in a variety of responses. Evaluation of TCDD in non-human primates revealed a decrease in ability to conceive and an increase in aborted fetus. Rhesus monkeys administered a continuous dose of 50 ppt TCDD in the feed (total dose 1.8 ug TCDD approx equivalent to 8.6 ng TCDD/kg/day) had increases in serum estradiol and progesterone. Abnormal hormone levels may have been responsible for only 2/8 treated animals conceiving versus 8/8 in the control group (Allen et al. 1979). McNulty 1985 reported a dose-related increase in fetal loss at <u>much higher levels</u>; 1.0 ug TCDD/kg and 5.0 ug TCDD/kg total dose (administration divided into 9 intragastric doses GD 20-40) which was accompanied by maternal toxicity. No hormone levels were measured in these Rhesus monkeys so its relationship to fetal loss can not be determined.

The effects of TCDD mediated alteration in hormone levels is not restricted to the reduced

ability to conceived or fetal loss. A decrease in uterine and body weight was observed in prepubescent female guinea pigs, rats and hamsters administered a single 4, 50, 400 ug TCDD/kg (an equitoxic) dose of TCDD (Hruska and Olson 1989). A statistically significant decrease in uterine weight occurred in guinea pigs and Sprague Dawley rats. Only a slight (not significant) decrease in uterine weight was observed in the hamster. In addition to uterine weight changes, increases in the uterine estrogen receptor density (measured using saturation analysis, followed by estimation of receptor density [Bmax] from Scatchard plot) were observed at above administered concentrations. The largest change in uterine ER density (Bmax) was in the rat, followed by the hamster, then the guinea pig. Neither the change in uterine weight nor the constitutive levels of ER (Bmax) predicted the increase in uterine receptor density. At these doses TCDD decreased the hepatic ER concentration significantly in rats and guinea pigs but not in hamsters. The species differences in TCDD mediated changes in hepatic ER may be partially explained by the constitutive levels of these receptors. In the three species studies, the constitutive level of ER in the liver predicted the magnitude of the decrease in hepatic ER. The differences in uterine and hepatic ER responses suggests that there may be other factors not found in the liver, but present in the uterus that modulate this response.

Species differences in many endpoints can be directly related to their differences in hepatic enzyme (P450) induction. Lin et al. (1991) investigated this relationship as it pertained to ER using C57BL/6 responsive strain (Ah^{bb}) and the non-responsive strain (Ah^{dd}). Mice were administered 0, 0.3, 1, 3, 10, 30, 100, and 300 ug TCDD/kg single oral dose. The study found that the magnitude of the decrease in hepatic ER in the Ah^{bb} mice was significantly more than that of the non-responsive mice. Although the dose (LOAEL) at which the decrease in hepatic ER was

observed was similar in both strains, approximately 0.06 ug TCDD/kg.

In intact and ovariectomized Long Evans rats (Romkes et al. 1987) a dose dependent decrease in hepatic and uterine ER was observed after administration of 20 ug TCDD/kg or 80 ug TCDD/kg TCDD. This study also showed that the administration of estradiol with the TCDD further decreased the ER in the uterus, but did not have an effect on liver ER. This again indicates other factors (primarily hormonal) are responsible for the increase in sensitivity of the uterus to TCDD.

The receptor mediated toxicity of TCDD has been discussed in detail in this document (Whitlock chapter). Clearly TCDD's effective concentration and mechanism of toxicity are comparable to that of nuclear hormones. This does not imply that there is a direct interaction between TCDD and hormone receptors. In a study in which female Long Evans rats were administered a single i.p injection of 0, 20, 40, and 80 ug TCDD/kg a dose-dependent decrease in the hepatic and uterine ER and progesterone receptors (PR) was observed (Romkes and Safe 1988). This study also demonstrated that TCDD has no binding affinity for the ER and PR which suggests that TCDD does not exert its antiestrogenic properties through competition with the ER or PR.

DeVito et al. (1992a) has recently demonstrated that down-regulation of the hepatic and uterine estrogen receptor (ER) occurs at doses that increase CypIA1 (Table IX) but there are no accompanying changes of serum estradiol or increases in 2-OH estradiol (DeVito et al., 1991). Graph E (using a Michaelis-Menten and a Power Law function) depicts the increase in hepatic Ah activity at doses that decrease hepatic estrogen receptor. Although the Michaelis-Menten function can be fit to this data it is the Power Law function that provides insight into this relationship. The

similarity in the apparent kinetic order ("g" value) for the percent decrease in hepatic estrogen receptor and percent increase in AHH activity suggests a common mechanism of action. Supporting this is the information that this TCDD induced down-regulation of the ER segregates with Ah receptor (Lin et al. 1991). Other investigators have suggested that, in the MCF-7 breast cancer cell line, that TCDD induced increases in P450 enzymes increase estrogen metabolism resulting in a decrease in the ER (Gierthy et al. 1988). This is significant because it demonstrates that TCDD may induce decreases in estradiol which are Ah receptor mediated, and suggests that clinical effects could result from a decrease in estradiol not directly from TCDD.

It has been shown that *c-fos* is a nuclear regulatory protein that plays a role in the control of estradiol induced cell proliferation (Astroff et al 1991). At very low i.p. administered doses (0.064, 0.128 ug TCDD/kg), reduced constitutive levels of uterine *c-fos* mRNA were observed in Sprague-Dawley rats, however coadministration of exogenous estradiol increased the levels of *c-fos mRNA* (Graph F-A). Although TCDD does not interact directly with ER, it may compete with the activated estrogen receptor at the level of transcription. The power law function applied to the TCDD induced decrease in *c-fos* resulted in a functional fit with $R^2 = 0.99$, "g" = 0.66 (Graph F-B). This is consistent with the "g" values described later in this chapter that were computed for the TCDD induced increase in EROD activity (Abraham et al, 1988, "g" = 0.66) and benzo(a)pyrene hydroxylase (Kitchin and Woods, 1979, "g" = 0.77), suggesting the mechanisms by which TCDD alters *c-fos mRNA* may be, in part, common with those mechanisms involved in P450 induction.

Table IX HORMONE EFFECTS: DOSE RESPONSE

Species	Dosing Regimen	Effect	Dose	Response				NOAEL	LOAEL	Reference
Female Long Evans Rats	single ip	Uterine % decrease cytosolic ER % decrease nuclear ER % decrease cytosolic PR % decrease nuclear PR	 0 ug/kg 20 50 80	C-ER 0 % 16.4 % 23.6 % 46.2 %	N-ER 0 % 12.4 % 32 0 % 68.3 %	C-PR 0 % 14 % 20 % 25 %	N-PR 0 % 3.5 % 17.9 % 72.9 %			Romkes and Safe, 1988
Female Long Evans Rats	single ip	Hepatic % decrease cytosolic ER % decrease nuclear ER % decrease cytosolic PR % decrease nuclear PR	 0 ug/kg 20 50 80	C-ER 0 % 4.0 % 7.5 % 54.3 %	N-ER 0% 12.3 % 45.5 % 26.9 %	C-PR 0 % 3.5 % 18.1 % 63.4 %	N-PR 0 % 21.7 % 27.5 % 64 %			Romkes and Safe, 1988
Female S-D rats	single ip	% decrease in c-fos mRNA units	0 ug/kg 0.016 0.064 0.128	0 % 20 % 44 % * 82 % *				0.016 ug/kg	0.064 ug/kg	Astroff et al., 1991
Mice CD-1	single ip dose	% decrease hepatic ER % decrease uterine ER % increase hepatic AHH	 0 ug/kg 1 3 10 30	H-ER 0 % 9.4% 14.1 % 23.4 % 46.9 %	U-ER 0 % 4.5 % 0 % 40.9 % 36.5 %	H-Ahh 0 % 116.6 % 333 % 467 % 400 %				DeVito et al., 1992a

Profound changes occur in biological systems as a consequence of minute changes in hormone levels. Much evidence exists that TCDD acts like a hormone, primarily because of receptor action and the low concentrations that are associated with effects, but also because of the types of systems affected. Many qualitative changes that occur as a consequence of TCDD administration are well documented; however, the dose-response relationships for many of these endpoints have not been evaluated. Dose-response assessments and an increase understanding of the interaction with other hormone systems is important and should be a focus of future research.

F. Male Reproductive Toxicity

The male reproductive system is adversely affected by TCDD and related compounds. A detailed review of TCDD induced male reproductive toxicity is presented in the Reproductive and Developmental Toxicity chapter of this document (Peterson). Some of the effects are described here to help understand their dose-response relationships and compare the specificity and sensitivity of this response to other endpoints.

Overtly toxic doses of TCDD that decrease body weight and feed consumption decrease plasma androgen concentrations, testis and accessory sex organ weights and alters spermatogenesis and testicular morphology when administered to postweanling male rats (Moore et al. 1985, 1989, 1991; Mebus et al. 1987; Morrissey and Schewtz 1989; Kleeman et al. 1990; Bookstaff et al. 1990). The decreases in circulating androgens by TCDD are mediated through at least two different pathways. TCDD inhibits testicular steroidogenesis (Kleeman et al. 1990) and alters the regulation

of pituitary luteinizing hormone secretion (Bookstaff et al. 1990). The ED_{50} for the androgenic deficiency is approximately 15 ug TCDD/kg (Moore et al. 1985). In this study Moore et al., administered single oral doses of 0, 6.25, 12.5 25, 50 and 100 ug TCDD/kg to male Sprague-Dawley rats. Serum testosterone was measured 7 days post administration. Graphic representation (Graph G) depict a dose-dependent decrease in serum testosterone. The Power Law function fit this data ($R^2 = 0.92$). When the power of the curve fit was compared to the power of the curve fit of other endpoints (Benzo(a)pyrene hydroxylase induction Graph K and EROD induction Graph M) it was found that the kinetic order ($g = \underline{0.60}$) was consistent with these other endpoints. There are no studies that can directly compare biochemical endpoints quantitatively to male reproductive endpoints because protocols similar to those designed by Moore et al. (1985) have not been used. However, the similarity of the curve fit using the power law function to biochemical endpoints (see biochemical endpoints discussion, section G) suggests a common mechanisms may be responsible for the decrease in serum testosterone. These data indicate that the adult male reproductive system toxicity may be mediated by similar mechanism but is not as sensitive to the actions of TCDD in that androgen deficiency occurs at doses that induce overt toxicity.

While the adult male rat requires overtly toxic doses of TCDD to produce an anti-androgenic affect, the developing male reproductive system is markedly sensitive to TCDD. Maternal doses as low as 0.16 ug TCDD/kg decrease anogenital distance of 1 and 4 day old male rats, delays testicular descent and decreases seminal vesicle and ventral prostate weights in rats. The organ weights were consistently decreased from the fetal stage into adulthood (Mably et al. 1992a) Table X. Decreases in masculine sexual behavior and increases in feminization of male rats

also occurred at maternal doses of 0.16 ug TCDD/kg (Mably et al. 1992b), in addition spermatogenesis was altered at maternal doses as low as 0.064 ug TCDD/kg. The single administration of 0.064 ug TCDD/kg to pregnant rats on gestational day 15 consistently decreased epididymis and cauda epididymis weights, cauda epididymal sperm counts and daily sperm production (Mably et al. 1992c). None of the doses of TCDD resulted in any demonstrable overt maternal toxicity (Mably et al. 1992a,b,c). These data indicate that in utero and lactational exposure to TCDD permanently impaired the development of the male reproductive system, as well as possibly impairing sexual differentiation of the CNS. Hence, the developing male rat reproductive system is one of the most sensitive to the toxic effects of TCDD.

The mechanism(s) by which in utero and lactational exposure to TCDD alters the development of the male reproductive system is not completely understood. Decreases in circulating androgens are mediated in part by reductions in testicular responsiveness to luteinizing hormone (LH) stimulation (Mably et al. 1992a). Decreases in accessory sex organ weights may be due to a combination of decreased androgen levels and decreased responsiveness of these organs to androgens (Mably et al. 1992a). Future studies are indicated to determine the relative importance of these effects, and of the possible role of altered testosterone metabolism in response to TCDD induction of phase I and phase II enzyme systems.

The alterations in sexual behavior in male rats appears to be due to impairment of sexual differentiation of the CNS which requires the presence of testosterone during perinatal development for male differentiation (Mably et al. 1992b). Once in the CNS, testosterone is converted to estradiol and both of these steroids must bind to their respective receptors for the proper

Table X Male Reproductive Toxicity

Species/Strain	Dosing Regimen	Effect	Dose	Response % decrease			NOAEL	LOAEL	Reference
Holtzman Rats	single dose GD 15	seminal vesicle wt male offspring after birth	0.0 ug/kg 0.064 0.16 0.4 1 0	0% day 32 0% 14% 27% 32%	0% day 49 9% 13% 48% 65%	0% day 63 13% 28% 38% 44%			Mably, 1992a
Holtzman Rats	single dose GD 15	ventral prostate weight percent of control	0.0 ug/kg 0.064 0.16 0.4 1.0	0% day 32 21% 29% 43% 57%	0% day 49 +9% +6% 25% 50%	0% day 63 19% 31% 44 47%			Mably, 1992a
Holtzman Rats	single dose GD 15	Caudal epididymis wt in male offspring	0.0 ug/kg 0 064 0.16 0 4 1.0	0% day 32 17% 21% 42% 46%	0% day 120 7% 10% 20% 25%				Mably, 1992a
Holtzman rats	single dose GD 15	anogenital distance/crown rump length day 1 or 4 after birth	0.0 ug/kg 0.064 0.16 0.4 1.0	0% day 1 6% 18% 29% 29%	0% day 4 9% 18% 27% 23%				Mably, et al., 1992a
Holtzman Rats	single dose GD 15	Caudal epididymal sperm in offspring	0.0 ug/kg 0.064 0.16 0.4 1.0	0% day 63 41% 53% 65% 71%	0% day 120 33% 37% 48% 58%				Mably, 1992c
Sprague Dawley rats	single po dose	serum testosterone level reported as % decrease	0.0 ug/kg 6.25 12.5 25 50 100	0% 18.8% 37.5% 43.8% 93.8% 93.8%					Moore, et al., 1985

differentiation of the CNS. Maternal doses of 1 ug TCDD/kg decreased plasma testosterone levels in male offspring on days 18 through 21 of gestation (Mably et al., 1992b). The reduction in plasma testosterone may account for the demasculinization and feminization of male rats seen in adulthood. However, it is possible that TCDD alters the sensitivity of the developing pituitary to androgens and estrogens (Mably et al. 1992b) perhaps by altering the respective hormone receptors (Umbreit and Gallo 1988; Devito et al 1990). The mechanism of the altered sexual behavior of male rats exposed in utero to TCDD remains undetermined. Quantitative dose response analysis of these very sensitive effects in male offspring are complicated by the incomplete understanding of the toxicokinetics of TCDD in the fetus. Additional research that focuses on understanding tissue distribution and metabolism in the fetus will aid in comparative analyses and help to better understand the implications of these findings for a human population.

G. Liver Toxicity

Hepatotoxicity is consistently found in animals across species, administered TCDD. The liver is one of the major sites for metabolism of exogenous and endogenous chemicals. Although clinical and morphological changes are seen in the liver, it is the induction of hepatic cytochrome P450IA1 family of metabolizing enzymes that is one of the most sensitive indicators in many species for receptor mediated responses.

Guinea pigs are most sensitive to the acute lethal effects of TCDD. Harris et al.(1973) administered 0, 0.008, 0.04, 0.2, and 1.0 ug TCDD/kg weekly for 8 weeks (equivalent to 0, .001 .006, .029, and 0.143 ug TCDD/kg/day) (Table XI). Absolute liver weight was not a sensitive indicator of toxicity, although liver to body weight ratios calculated from graphic data do show an increase at the

highest dose. DeCaprio et al. (1986) described the effect of subchronic exposure to TCDD on guinea pigs. Animals were administered 0, 2, 10, 76, and 430 ppt TCDD in feed for 90 days. Equivalent total doses calculated by author were for males 0.0, 0.12, 0.61, 0.4.9 and 26 ng/kg/day and for females 0.0, 0.12, 0.68, 4.9, and 31 ng/kg/day. At the high dose 60% of the male and female animals either died during study or were sacrificed in a moribund condition. A significant increase in liver to body weight ratio was observed in males and females at the 4.8 and 4.86 ng/kg/day level. A dose dependent trend of increased relative liver weight was observed. These changes were not accompanied by other signs of hepatic toxicity. It is interesting to note that the female guinea pig is more sensitive to the chronic effects of TCDD than to the acute toxic effects. This was also observed in S-D rats in the Kociba et al. (1978) chronic toxicity study.

Hamsters are relatively insensitive to the acute toxic effects of TCDD, although the response to TCDD in the liver is qualitatively consistent with other species. A dose-dependent increase in absolute liver weight was observed in hamsters 50 days after administration of a single i.p. injection of 0, 5,25,100, 500, 1000, 2000, and 3000 ug TCDD/kg (Table XI). Graphing the Olson et al. (1980) data using a Michaelis-Menten function resulted in a very poor fit (Graph H). These data are extremely variable which may be due to the timing of the measurement in relation to the $T_{1/2}$ of TCDD in hamsters. Peak liver concentrations of TCDD are reached at 3 days in hamsters, therefore liver weight measurements taken at 50 days may not accurately reflect tissue toxicity (Olson et al. 1980a; Gasiewicz 1979).

To assess the hepatoxic effects of TCDD on C57BL/6J mice Vos et al.(1974) administered male mice weekly doses of 0, 0.2, 1.0, 5.0 and 25.0 ug TCDD/kg. Mice were killed at 2 and 6 weeks to assess the systemic effect of TCDD. The relative liver weights generally increased with increasing

dose. A statistically significant increase was seen at 2 weeks and at 6 weeks at the 1.0 ug/kg dose level (equivalent to 0.14 ug TCDD/kg/day)(Table XI). Hepatic damage was characterized as degeneration of liver cells around the central vein and proliferation of bile duct epithelial cells (at 25 ug TCDD/kg). At the 5 ug TCDD/kg level, necrotic hepatocytes and inflammatory cells were noted in the centrilobular region. At the lowest dose 0.2 ug TCDD/kg (equivalent to 0.28 ug TCDD/kg/day) centrilobular fatty change was noted. Fatty change increased in severity with increasing dose. Microscopic observation of the liver was the most sensitive indicator of frank hepatic toxicity following TCDD exposure.

Shen et al. (1991) investigated the role of TCDD in liver injury in C57Bl/6J and DBA mice administered a single dose of 0, 3, and 150 ug TCDD/kg and 30, and 60 ug TCDD/kg respectively (Table XI). Increased liver/bwt ratio and decreased body weight were the most sensitive indicators of toxicity. On day 7 the C57BL/6J mice had an equivalent increase in relative liver weight at 1/10th the dose required to elicit the same response in DBA mice, however too few dose levels were used to assess a dose response relationship. Changes in liver/bwt ratio are a good surrogate for histopathological changes in the liver of these mice however, in guinea pigs this relationship was not found. In the DBA mouse no fatty change was observed at 30 ug TCDD/kg, but mid-zonal fatty change was noted at 600 ug TCDD/kg at 7 d. Focal necrosis was noted at 30 ug TCDD/kg 7 d and at 600 ug TCDD/kg at 3 and 7 days post dosing. In the C57BL/6J mouse fatty changes were noted at 3 ug TCDD/kg at 3 and 7 days, and severe diffuse fatty changes were noted at 150 ug TCDD/kg at 3 and 7 days. Focal necrosis and focal acute and chronic inflammation was observed at 150 ug TCDD/kg at 1 and 3 days post-dosing. (Shen et al. 1991) Hence, the sensitivity for necrosis and fatty change is again approximatley 10-fold between the C57BL/6J and the DBA.

The differences in TCDD sensitivity between C57BL/6J and DBA mice is believed to be mediated in part at the level of the Ah receptor gene locus. Poland and Knutson (1982) hypothesized TCDDs mechanism of toxicity as TCDD binding to an endogenous cytosolic protein (Ah receptor) in a manner steroid hormones bind to their receptors. This receptor-ligand complex interacts with DNA to regulate transcription of several proteins, including the cytochrome P-450IA1 which is assayed as the enzyme aryl hydrocarbon hydroxylase (AHH). TCDD in not mutagenic and these DNA interactions do not damage DNA. To further clarify the relationship between receptor binding and toxicity, Birnbaum et al. (1990) used C57BL/6J $Ah^{d/d}$ congenic "resistant" mice and wild type $Ah^{b/b}$ "sensitive" mice. A single dose of 0, 400, 800, 1600, 2400, and 3200 ug TCDD/kg TCDD was administered to $Ah^{d/d}$ mice and 0, 50, 100, 200, 300, and 400 ug TCDD/kg TCDD to $Ah^{b/b}$ mice. Relative liver weights were significantly increased at all doses. This is expected since doses were much higher than the dose administered by Shen et al. 1991 in which 3 ug TCDD/kg that resulted in increased relative liver weights. The severity of a variety of liver lesions were compared in the congenic and wild type mice. Hepatocellular cytomegaly and hepatocellular karomegaly occurred at 8 times the dose in $Ah^{d/d}$ mice compared to $Ah^{b/b}$ wild type mice. The differences were greater for fatty change in the liver and bile duct hyperplasia. Qualitatively the toxicity seen in these two strains of mice were similar; but the doses that result in this toxicity differ by 8-24 times. (Birnbaum et al. 1990) In male C57BL/6J mice administered single oral doses of 95, 145, and 190 ug TCDD/kg, a dose-related increase liver, and liver/bwt ratio was observed. In male DBA mice administered a single dose of 1370, 1870 and 2610 ug TCDD/kg TCDD an increase in absolute and relative liver weight was observed at every dose, but not with a dose response trend (Chapman and Schiller 1985). These same authors reported a dose-dependent decrease in serum glucose, total serum cholesterol,cholesterol ester,

serum triglycerides, and an increase in liver triglycerides in both strains of mice. The dose response data is reported graphically.

Pohjanvirta studied two strains of rats, Long Evans (L/E) a species sensitive to TCDD with a LD_{50} of 10 ug TCDD/kg and the Han/Wistar (H/W) rat with a LD50 of >3000 ug TCDD/kg.(Pohjanvirta 1990). In assessing target organ toxicity the L/E rats received a single ip injection of 5, and 50 ug TCDD/kg. The H/W rats received an additional dose of 500 ug TCDD/kg. Relative organ weights were assessed on day 1,4,8,16 and 39-40. The only difference observed in relative liver weights was the initial statistically significant increase on day 1 in the H/W rats at the two highest doses. Both species were observed to have a statistically significant increase in relative liver weight on day 3 to day 16. There were marked strain differences observed histopathologically. In L/E rats 50 ug TCDD/kg (5 X LD_{50}) four days post-dosing resulted in multinucleated and swollen hepatocytes. Only minor lesions were observed in H/W rats administered up to 500 ug TCDD/kg (Pohjanvirta 1989). Distribution of TCDD to the liver did not account for any differences in liver toxicity. Both strains had peak TCDD liver concentrations on day 8 and the rate of liver tissue disappearance appeared equivalent. This is unlike the thymus distribution in which was qualitatively very different between these two strains (Pohjanvirta 1990).

A dose-dependent increase in relative liver weight was observed in male and female Sprague-Dawley rats (Kociba et al. 1976) administered 0.001, 0.01, 0.1, 1.0 ug TCDD/kg/day 5 days/week for 13 weeks (Equivalent doses: 0.0007, 0.007, 0.07, 0.7 ug TCDD/kg/day). Liver to body weight ratios (Table XI) were increased in males at the 0.1 and 1.0 ug TCDD/kg dose level. Female rats were more sensitive, having a statistically significant increase in relative liver weight additionally at the 0.01 dose level. This is illustrated using the Power Law function (Graph I). The R^2 values for males and females (0.93, 0.94) are high for the Power Law indicating a good fit to the data.

Table XI Liver Weight Changes

Species/Strain	Dosing Regimen	Effect	Dose ug TCDD/ kg - Response			NOAEL	LOAEL	Reference
Hartley Guinea Pigs female	TCDD administered orally 1 X week for 8 weeks	increase absolute liver weight increase liver/bwt estimated from graph	0 ug/kg 0 ug/kg/day 0 008 001 0 04 006 0 2 029 1 0 143	0 % change liver wt. +0 3% -8 5% -8 5% N/A	0% change liver/bwt +4% -2% +14 3% N/A			Harris, 1973
Hartley Guinea pigs (230-340) Males	oral continuous feed 90 days	increase liver/bwt reported as % control (total dose reported)	0 ug/kg 0 ng/kg/day 0 011 0 12 ng/kg/day 0 055 0 61 ng/kg/day 0 441 4 9 ng/kg/day	0 -9 7% (decrease) +18.1% +24.0%*		0 055 ug/kg	0 44 ug/kg	DeCaprio, 1986
Hartley Guinea pigs (230-340) females	oral continuous feed 90 days	increase liver/bwt reported as % control (total dose reported)	0 ug/kg 0 ng/kg/day 0 011 0 12 ng/kg/day 0 061 0 68 ng/kg/day 0 437 4 9 ng/kg/day	0 +4.4% -0.6% (decrease) +28%*		0 061	0 44	DeCaprio, 1986
Rhesus Monkeys <70 kg	single oral gavage	increase liver as percent bwt 4-6 wks	0 70 350	0 45 8% * 47.0 % *			70 ug/kg Lowest dose tested	McConnell, 1978
Female syrian golden hamster 51-60 g	single i p injection	change liver weight 50 days	0 ug/kg 5 25 100 500 1000 2000 3000	0 -5.3% +19% +8.6% +22.1%* +42%* +27.4%* 4.9%		100 ug/kg	500 ug/kg	Olson, Holscher, and Neal, 1980
C57BL/6J male mice	weekly doses p o	increase liver/bwt ratio reported at (2 weeks) and (6 weeks)	ug/wk (2) (6) 0 (0) (0) 0 2 (4) (1 2) 1 0 (2) (6) 5 0 (10) (30) 25 (50)(150)	0 1 3% 11.2%* 15 5%* 17.5%*	0 6.8% 17.6%* 26.4%* 42.6%*	0 4 2 weeks 1 2 6 wks	2 0 -2 wk 6 0-6 wk	Vos, 1974
C57BL/6J male mice 20-28 g	single i p in olive oil	increase liver/bwt ratio as % control 1, 3, 7 days post-dosing	0 dose 3 150	0 day 1 -5% -2%	0 day 3 +3.6% +11%*	0 day 7 +22%* +41%	3 - 3 days N/A -7 days	150 - 3d 3 - 7d	Shen, 1991
DBA male mice 20-28g	single i p in olive oil	increase liver/bwt ration as % control 1, 3, 7 days post dosing	0 ug/kg 30 ug/kg 600 ug/kg	0 day 1 -5.8% +9.8%*	0 day 3 0 0	0 day 7 +11 5%* +27%*	30 ug/kg 1,3 days	30 ug/kg - 7d	Shen, 1991
C57BL/6J male mice DBA male mice	single p o	increase liver/bwt ratio 30 days post dosing	0 ug/kg C57BL 0 DBA 95 1370 145 1870 190 2610	0 C57BL 13.9%* 32%* 41.7%*	0 DBA 53% 36.8% 46.5%		N/A	95	Chapman and Schiller, 1985
		increase liver/bwt ratio reported as ug/kg/day and (total dose)	0 (0) ug/kg 0 001 (0 065) 0.01 (0.65) 0 1 (6.5) 1 0 (65)	0 (measured dose) N/A 0 0037 ug/g liver 0.0346 0 2840	0 3 2% 4 5% 22% 35%	0 065 ug/kg	0 65 ug/kg	Kociba et al., 1976
Sprague-Dawley rats males	p o 5 x/wk 13 weeks	increase Liver/bwt ratio reported as ug/kg//day and (total dose)	0 (0) ug/kg 0 001(0 065) 0 01 (0 65) 0 1 (6 5) 1 0 (65)	0 (measured dose) N/A 0.0026 ug/g liver 0 0360 ug/g liver 0.3240 ug/g liver	0 3.2% 4.5%* 22%* 35%*	0 065 ug/kg	0 65 ug/kg	Kociba et al ,, 1976

The "g" values for these functions indicate a sublinear dose response. Attempts to fit the Michaelis-Menten function resulted in a very poor fit suggesting that a sigmoid dose-response may not characterize this phenomenon. The female rats were also more sensitive than male rats as measured by histopathology, clinical chemistry and relative liver weight. Microscopic examination revealed some hyperplasia in the bile ducts and altered hepatocytes at the 1.0 ug TCDD/kg dose level of both sexes, characterized as multinucleated and irregularly shaped cells, lipid accumulation, and some necrosis. Slight changes in hepatocytes "variations in size and shape" were noted in the 0.1 ug TCDD/kg level. No abnormalities were noted microscopically in the 0.001 or 0.01 dose levels. Clinical chemistry also revealed a statistically significant increase in total bilirubin, direct bilirubin, and alkaline phosphatase at the two highest doses in females (0.1 and 1.0 ug TCDD/kg) and in the highest dose in males. Increase relative liver weight was the most sensitive indicator of liver toxicity in this study. This study was terminated at 13 weeks at which time a parallel study reported that steady state concentrations of TCDD are found in the fat and the liver (Rose 1976).

G. Biochemical Endpoints

The mechanism of TCDD toxicity involves the formation of a receptor-ligand complex which results in an increase in the transcription of P450 liver enzymes. The increase in enzyme production may account for the increase in liver weight, and may also be responsible for other toxic endpoints.

Cytochrome P450 is a family of enzymes which catalyze the metabolism of a wide range of endogenous lipophilic compounds (steroids, fatty acids, cholesterol derivatives) as well as a number of xenobiotics. There is a large body of literature which point to major differences between

different animal species, and humans when comparing specific cytochrome P450 forms and substrate specificity. These differences are also important when comparing sexual differences and age related effects in animals. The differences in species, sex and age sensitivity can be attributed to which cytochrome P450s are constitutively expressed and the inducibility of all isozymes. If the mechanism of toxic effects are mediated by the cytochrome P450 then the differences observed between animals can be explained. The different responses are probably due to a number of factors, because of the complexity of the biological response. As outlined in the Whitlock chapter (this document), the effects caused by dioxin interaction with the receptor are a multi step process which, with alterations at any of the steps, will alter the response. In graphing the response of activity in a single species there is very good agreement for the levels of Ah receptor in the cytosol and nucleus, the mRNA for P450IA1 and the induction of enzymatic activity using EROD as the substrate in a single tissue. Tissue sensitivity may differ because of the presence of tissue specific stuctural orthologs of P450. (Wrighton, 1990; Nebert et al. 1989). There can be differences between species and sex as to which forms are constituitively expressed and there inducibility. There may also be species specific forms, which make a direct extrapolation to man difficult (Guengerich 1989). The presence or absence of a specific isozyme in a tissue or cells within a tissue will also effect the toxicity. As stated above and discussed in this document (Witlock chapter), the multi step process of induction of the TCDD responsive enzymes is mediated through a cytosolic receptor. The differences in the receptor and the regulation of the genes and post-translational events between species and sex can result in distinctly different toxic responses. The importance of the genetics in the effects caused by TCDD is best demonstrated in the Ah responsive and nonresponsive mice. In the homozygous mouse the animals are very sensitive to

many TCDD mediated responses, while the heterozygous mouse strain is less sensitive.

The P450I family is present in rodents and humans and the family contains these two members Cytochrome P450IA1 (CypIA1) and P450IA2 (CypIA2). Both of these forms are important in the response observed following TCDD treatment. The CypIA1 is an ortholog of the rat P450c (also known as P448). In mammalian species this can be measured as aryl hydrocarbon hydroxylase (AHH) and is expressed at very low levels in the liver and extrahepatically. In animal studies this is the major form that is induced following polyaromatic aromatic hydrocarbon treatment. Wrighton (1990) has reported that in man P450IA1 is rarely expressed in human liver and is predominantly an extrahepatic form, and as such is inducible by cigarette smoke and polyaromatic hydrocarbons (PAHs).

The second member of this family is CypIA2, which is present the untreated animals constitutively at levels greater than CypIA1, is inducible by polyaromatic hydrocarbons(PAHs) (Sesardic et al 1988; Quattrochi and Tukey 1989). The human CypIA2 (HLd) is expressed in all human livers examined. This is important because this isoform is believed to be a specific (non receptor) binding protein in hepatic microsomes, which may play an important role in the human response to TCDD (Wrighton 1989). Unlike the CypIA1 which in the human liver is not inducible, the CypIA2 is inducible by constituents of cigarette smoke and other sources of polyaromatic hydrocarbons. In animal studies the CypIA2 is inducible following TCDD exposure.

Hook et al. (1975) characterized the induction of microsomal mixed-function oxidases (MFO) and cytochromes following TCDD treatment. There was a decrease in N-demethylation enzyme activity in the liver of female C-D rats not seen in male rats; however, there was no difference in the induction of P450° or benzo(a)pyrene hydroxylase. In addition, Hook in an

enzyme assay using tissues from male rats administered 25 ug TCDD/kg found there was liver and kidney induction of biphenyl 4-hydroxylase, biphenyl 2-hydroxylase, benzo(a)pyrene hydroxylase, UDP-glucuronyl transferase and benzphetamine N-demethylase. In the lungs, intestine, brain and testes of these animals there was no induction of biphenyl 4-hydroxylase, and biphenyl 2-hydroxylase, but there was a significant induction of all other enzymes. In guinea pigs dosed with 0.175 ug TCDD/kg biphenyl-4-hydroxylase was reported in liver, lung, and kidney tissues 4 days after administration. In rabbits administered 25 ug TCDD induction of 4-hydroxylase was observed in lung tissue and not in kidney and liver. Induction of benzo(a)pyrene hydroxylase was observed in kidney and liver, but not in the lung. These differences are difficult to interpret because only one dose was administrated, but suggested definite species, sex and tissue differences in response to TCDD. The clinical manifestations of these enzyme changes are not well understood, but is a focus of current research.

To understand the tissue differences in enzyme induction, EROD; ethoxyresorufin dealkylase activity a measure of CypIA1 activity was quantified in the homogenized lung and liver of male Swiss mice after ip injection of 5 or 50 nmol/kg TCDD. Results indicate that the induction of P450 IA1 in the liver and lung follow a very similiar pattern. Both liver and lung have an immediate and significant increase in EROD 15 fold and 25 fold respectively over the very low consituitive levels (Beebe et al. 1990). Comparing the EROD activity in the lung administered 50 nmol/kg versus the liver administered 5 nmol/kg reveals that the liver has 5 times the activity (40 nmoles resorufin/min/g liver vs. 8 nmoles/g lung). These differences may be accounted for by differences in tissue distribution, as Gasiewicz et al. (1983) reported an approximately 7 to 10 fold difference in TCDD distibution to the liver vs lung in the three species of mice in which measurements have been made.

Kitchin and Woods (1979) evaluated a dose response relationship between TCDD and the elevation of the liver enzymes as total p-450 and benzo(a)pyrene hydroxylase in microsomes. Additionally, they administered 2 ug TCDD/kg to rats and examined the increase enzyme activities associated with a number of cytochrome p-448 type substrates (biphenyl 2-hydroxylase, biphenyl 4-hydroxylase, 4,4'dimethyl-biphenyl hydroxylase, benzo(a)pyrene hydroxylase, EROD, and benzphetamine N-demethylase). All but the last enzyme was elevated significantly by an exposure of 2 ug TCDD/ kg. In a third experiment, Kitchin and Woods (1979) administered a single po dose of 2 ug TCDD/kg to female S-D rats and reported EROD, cytochrome P-450, and AHH levels 1, 3, and 6 months post-dosing. Results indicate that enzyme remain elevated even after 6 months although the levels of AHH and EROD are substantially reduced after 6 months. Kitchin and Woods (1979) do some interesting calculations based on TCDD radiotracer experiments. TCDD levels in the liver, thymus and adipose 3 days post-dosing of 2 ug TCDD/kg resulted in tissue concentrations of 1.08, 0.37, and 1.84% of oral dose per gram of tissue (wet weight). They determined that as few as 65 molecules of TCDD in a hepatocyte can significantly increase the enzyme activity of Benzo(a)pyrene hydroxylase. (Table XII). Both total P450 and BAP hydroxylase fit the Power Law functional form with high correllation, but have different kinetic orders (g = 0.58, 0.42) which may indicate different factors regulate each (Graphs J and K).

Abraham et al. 1988 described the response of hepatic ethoxyresorufin-O-deethylase (EROD) and total P450 induction in female Wistar rats (See Table XII). Rats were administrated doses ranging from 1 to 3000 ng TCDD/kg via subcutaneous injection. Subcutaneous absorption was reported to be slow, but almost complete. TCDD induced a significant increase P450 activity at 0.1 ug TCDD/kg and in EROD at 0.3 ug TCDD/kg. The EROD response continued to increase

enzyme assay using tissues from male rats administered 25 ug TCDD/kg found there was liver and kidney induction of biphenyl 4-hydroxylase, biphenyl 2-hydroxylase, benzo(a)pyrene hydroxylase, UDP-glucuronyl transferase and benzphetamine N-demethylase. In the lungs, intestine, brain and testes of these animals there was no induction of biphenyl 4-hydroxylase, and biphenyl 2-hydroxylase, but there was a significant induction of all other enzymes. In guinea pigs dosed with 0.175 ug TCDD/kg biphenyl-4-hydroxylase was reported in liver, lung, and kidney tissues 4 days after administration. In rabbits administered 25 ug TCDD induction of 4-hydroxylase was observed in lung tissue and not in kidney and liver. Induction of benzo(a)pyrene hydroxylase was observed in kidney and liver, but not in the lung. These differences are difficult to interpret because only one dose was administrated, but suggested definite species, sex and tissue differences in response to TCDD. The clinical manifestations of these enzyme changes are not well understood, but is a focus of current research.

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even at doses 1000 times this. No maximum induction was reached in this study. The Michaelis-Menten and Hill plots graphically depict this phenomenon (Graph L and M). TCDD liver concentrations continued to increase at concentrations up to 3 ug TCDD/kg. This was attributed to steady state not being reached at day 7, but maximum-induction being reached at higher doses should not be discounted based on this data.

Experiments by Abraham et al. (1980) and Kitchin and Woods (1979) (Table XII) provided sufficient doses to further analyze the four functional forms described in the introduction. Kitchin and Woods (1979) measured benzo(a)pyrene hydroxylase (aryl hydrocarbon hydroxylase - AHH) induction and total P450 concentration in the liver of female Sprague-Dawley rats 3 days after TCDD administration. Abraham et al. (1980) used Wistar rats and measured EROD and total hepatic P450 at 7 days post dosing.

For the Kitchin and Wood (1979) data the linear function of dose-response was not an appropriate fit (Graph J1-A) for the total P450 (R^2 = .67). The Michaelis-Menten fit of raw data (J1-B) depicts an "inertial response" in the dose range from 0.0006 to 0.06 ug TCDD/kg dose range and appears to reach maximim at 20 ug TCDD/kg indicating that this function does describe the data. The Hill function (Graph J1-C) also fits the data points with a sigmoid curve and an exponent of 1.09. A Hill exponent of 1.0 is generally interpreted as having a linear dose response at low doses without cooperativity. However, the validity of this interpretation comes into question because the x-y plot does not depict linearity at low doses. The Power Law fit, over the entire dose-range (Graph J2-A), resulted in a "g" = 0.76, (R^2 = 0.98) suggesting a sublinear dose-response. Systematic application of the Power Law resulted in a sublinear response in the low dose range (0.0006 to 0.6 ug TCDD/kg) (Graph J2-B]) with a "g" = 0.035; R^2 = 0.51 which increased at high

dose range to 0.11, R^2 = 0.97. This shift of "g" values form 0.035 to 0.11 or greater defines the "inertial response". Graphs L depicts the Abraham et al. (1980) P450 raw data. The Hill function applied the Abraham et al.(1980) P450 raw data has an exponent of 0.55 (sublinear indicating negative cooperativity in the low dose range, while Kitchin and Woods (1979) had an exponent of 1.09. In addition the fit to the Michaelis-Menten function does not appear to reach a maximal response (GraphJ1-B) indicating this function does not describe the dataset.

It is the Power Law function that suggests a consistent response between these two datasets. The change in kinetic order between the low doses (g = 0.036, R^2 = .88 [Abraham et al., 1980); (g = 0.035, R^2 =0.51; [Kitchin and Woods 1979]) and high doses (g = .14, R^2 = .99 [Abraham et al. 1980]); (g = 0.11, R^2 = 0.97; [Kitchin and Woods 1979]) is demonstrated with an improved "goodness of fit".

Total P450 is a non-specific measure of response and may not be a good predictor of clinical toxicity. The induction of enzyme activity is a measure of a specific post-transcriptional event and as such may serve as a more consistent predictor of clinical outcome. Therefore, the induction of benzo(a)pyrene hydroxylase was analyzed (Kitchin and Woods 1979). The Michaelis-Menten and Hill functions both graphically depict an inertial response at low doses and a maximum response a high doses (Graph K1). The exponent for the Hill function is 1.07 (compared to 1.09 for the P450 induction) suggesting that both responses may be mediated by similar mechanisms. Systematic analysis of subsets of doses (Graph K2-B) resulted in a comparable trend of increasing kinetic order with increased dose range. The low dose (0.0006 to 0.004 ug TCDD/kg) kinetic order (g value) was 0.21, R^2 = 0.96 which increased with doses in the 0.02 to 0.6 ug TCDD/kg range (g = 0.77, R^2 = 0.96. In the highest dose range tested (0.6 to 20 ug TCDD/kg)

the kinetic order decreased to 0.19, $R^2 = 0.80$.

Similar responses were observed when EROD activity was measured. Graphs M depicts the functional forms used in this analysis. Both Michaelis-Menten(Graph M1-B) and Hill functions (Graph M1-C) graphically depict a low dose "inertial" response. The Hill function has a exponent of 1 which is consistent with the total P450 response (indicating no cooperativity and suggesting linearity in low dose range). The Power Law functional fit on all data points resulted in a sublinear fit, g= 0.52, $R^2 = 0.95$ (Graph M2-A). Analysis of subsets of data resulted in a response consistent with the Kitchin and Woods (1979) data analysis, ie., an increase in kinetic order from the low dose range (g = 0.11) to the high dose range (g = 0.66). For EROD induction the "Goodness of fit" for these both subsets of data was high, R^2 =0.99(Graph M2-B). The analysis of these two datasets (Kitchin and Woods, 1979; Abraham et al., 1980) suggests differences in the low and high dose responses. The Power Law function is commonly used to describe physical phenomenon, therefore the dose response fit of this model may be best described using laws of physics. The low dose response can be characterized as an "inertial" in which activity is measurable, but at a reduced rate. Not until an "external force" acts upon the system is an altered response (manifested as change in kinetic order) measured. The Power Law function when applied to total P450, EROD, and benzo(a)pyrene hydroxylase describes this phenomena.

In female mice (Narasimhan et al.(1993) see Table XIII) measured the individual steps of the multistep process of enzyme induction. Graphic representation of the total nuclear Ah receptor binding, mRNA for CypIA1, mRNA for Cyp1A2, and enzyme induction (EROD) plotted against dose demonstrated parallel slopes when fitted to a Power Law function. R^2 values indicate good fit to these functions (Graph N). This data supports the conclusion that the steps of this multistep

process are regulated by similiar mechanisms, and there is not likely to be post-translational modification for EROD induction. However, there is a difference in Cyp1A1 and Cyp1A2 mRNA levels.

The differential dose-response in induction of CypIA1 and Cyp1A2 has recently been investigated (Trischer et al. 1992). Female rats (initiated with DEN or saline) were administered TCDD biweekly p.o. for 30 weeks. Dose equivalents of 3.5, 10.7, 35.7, and 125 ng/kg/day resulted in a dose-dependent increase in hepatic CypIA1 and CypIA2 (measured by RAI). The rate of induction (different slopes) of the two enzymes differs only slightly and probably not significantly (Graph O). The differences in the Power Law function between these two enzymes is not as great as the difference detected by DeVito et al. (1992) (see below) for hepatic CypIA1 and CypIA2, possibly because of the different techniques of enzyme measurement used in this study; therefore definative conclusions can not be made. Additionally, maximum induction of Cyp1A1 is reached at a liver concentration of 10 ppb, wheras Cyp1A2 activity continues to increase after 30 weeks at hepatic concentrations of 30 ppb. Immunolocalization of enzyme induction in this experiment indicated that hepatocytes are not induced uniformily (Trischer et al. 1992). The mechanisms responsible for this differential induction may help in the understanding of intraspecies variability of response and prove useful in risk estimation.

The induction of skin, hepatic and lung EROD and hepatic 1A2 (acetanilide hydroxylase activity) was measured in female B6C3F1 mice administered 0, 1.5, 4.5, 15, and 150 ng/kg/day for 13 weeks (total doses 0, 0.098, 0.29, 0.98, 9.8)(DeVito et al. 1992 see Table XIV). Graphic representation of dose-response data indicates parallel slopes for all EROD induction, but a different slope for 1A2 induction (Graphs P1-P4). The fit to the Power Law functional form for EROD in

the three tissues measured had a high correllation coefficient. There was also a striking similiarity between the Power Law for the EROD measured in the three tissues versus the power law for hepatic CypIA2. The difference in induction between hepatic and lung EROD ranges from 5 fold to 18 fold which may be accounted for by differences in tissue distribution, although other factors can not be ruled until tissue dosimetry assays are run parallel to the enzyme induction assays.

Enzyme induction after a subchronic (13 week) exposure in B6C3F1 female mice was evaluated using the Devito et al.(1992) data. The linear regression model (Graph P1) for hepatic, skin and lung EROD induction had R^2 values of 0.95, 0.91, and 0.94 respectively; however, the R^2 for hepatic IA2 induction was 0.54. The Michaelis-Menten functional forms (Graph P2) graphically depict the "inertial" phase of the dose-response at doses less than 15 ng/kg/day (total dose = 0.975 ug TCDD/kg) for hepatic, skin and lung EROD activity. For hepatic EROD, the Hill function (Graph P3) exponent (1.1) was superlinear, whereas the exponent derived from application of the Hill plot for skin and lung EROD activity was sublinear (exponent = 0.83, 0.84). The "g" value in the Power Law function for hepatic, skin and lung EROD induction (Graph P4) depicts a sublinear kinetic order (g = 0.68, 0.75, and 0.79; R^2 = 0.92, 0.94, 0.97, respectively). These values are remarkably consistent, and interestingly are similar to the "g' values determined for EROD induction by Abraham et al., 1988 at a similar range of doses(at doses 0.3 to 3.0 ug TCDD/kg "g" = 0.66, R^2 = 0.99. Systematic analysis of subsets of data does not reveal the same change in kinetic order seen in the Abraham et al. (1988) or the Kitchin and Woods (1979) data sets; however, the total administered doses in the Devito et al. (1992) are not in the range of doses in which the change in kinetic order was observed. There is a notable increase in the "g" value for hepatic EROD induction (Table XV), but the R^2 values are low, and a consistent trend was not

observed. Although no change in kinetic order was observed in the lung and skin EROD induction, there are no acute data comparable to the Abraham et al. (1988) or Kitchin and Woods (1979) data for lung and skin induction. Therefore, a more complete understanding of the activity in extra-hepatic tissue is not possible with the available data.

The induction of P450IA2 was also measured (DeVito et al. 1992) using the functional forms previously described. The initial X-Y plot and linear plots of data revealed a much higher degree of variability in the data values. This observation is consistent with what has been found in humans populations, that P450IA2 constitutive values vary 3000% (Grant et al, 1983). This precludes making assumptions about the Hill or Michaelis-Menten plots.

TABLE XII DOSE-RESPONSE FEMALE RATS

Liver Enzyme Activity, Total P-450, Benz(a)pyrene, and EROD

Species	Dosing Regimen	Observed Effect	Dose ug TCDD/kg		Response	NOAEL	LOAEL	Reference
Female Sprague-Dawley rats	single oral dose	increase P-450 (3 days) (Omura and Sato, 1964)	0 0.0006 0.002 0.004 0.02 0.06 0.2 0.6 2.0 5.0 20.0		0.88 0% 0.91 3.4% 0.93 5.7% 1.0 13.6% 0.87 -1.1% 1.06 20.5% 1.16 31.8% 1.13 28.4%* 1.33 51.1%* 1.52 72.4%* 1.68 90.9%*	0.2	0.6	Kitchin, 1979
Female Sprague-Dawley rats	single oral dose	increase Benzo(α)pyrene hydroxylase (nmol polar products/mg protein/hr) (DePierre, 1975) at 3 days	0 0.0006 0.002 0.004 0.02 0.06 0.2 0.6 2.0 5.0 20.0		4.9 0% 4.9 0% 6.7 36.7%* 7.2 46.9%* 8.3 69.4%* 14.0 185.7%* 59.0 1104%* 96.0 1859%* 155.0 3063%* 182.0 3614%* 189.0 3757%*	0.0006	0.002	Kitchin, 1979
Wistar Rats Female	single s.c. injection	increase P-450 concentration (p-moles X mg protein^{-1}) measured as reduced CO difference spectra 7-days post dosing	0 ug/kg 0.001 0.003 0.010 0.030 0.100 0.300 1.00 3.00	0 (measured tissue dose) 0.0031 0.0102 0.0406 0.162 0.699 3.38 10.7 27.9	169 0% 182 7.1% 173 2.4% 184 8.9% 183 8.3% 199 17.8%* 230 36.1%* 269 59.2%* 319 88.8%*		0.100 ug/kg	Abraham,1988
Wistar rats Female	single s.c. injection	increase EROD Activity pmoles resorufin formed X mg protein-1 X min-1 (resorufin measured spectrofluorometrically in liver homogenates 7 days post-dosing)	0 ug/kg 0.001 0.003 0.010 0.030 0.100 0.300 1.00 3.00	0 (measured tissue dose) 0.0031 0.0102 0.0406 0.162 0.699 3.38 10.7 27.9	14.3 0% 16.9 18.2% 19.0 32.9%* 21.9 53.1%* 40.7 184.6%* 89.5 525.9%* 204.4 1329.4%* 495 3361.5%* 759.8 5213.3%*		0.003 ug/kg	Abraham,1988

TABLE XIII DOSE - RESPONSE B6C3F1 Female Mice
Liver EROD, Cyp1A1 mRNA, and Cyp1A2 mRNA and Total Nuclear Ah Receptor Binding

Species	Dosing Regimen	Effect	Administered Dose	Liver Dose fg/gm	Response		NOAEL	LOAEL	Reference
B6C3F1 female mice	single i.p. injection	24 hrs. post dosing increase EROD pmol/mg protein/min	0 ug/kg 0.005 0.010 0.025 0.05 0.10 0.50 1.00 5.00	0 0.11 0.10 0.09 0.26 0.74 2.36 11.5 53.16	0 +51% -8.0% -11% +9.0%* +109.6%* +252%* +538.5%* +1857%*	156 pmol/mg prot/min 235 168 139 170 327* 549* 996* 3053*	0.050 ug/kg	0.10 ug/kg	Narasimhan et al., 1993
B6C3F1 female mice	single i.p. injection	24 hrs post dosing increase relative mRNA units Cyp1A1	0 ug/kg 0.005 0.010 0.025 0.05 0.10 0.50 1.00 5.00	0 0.11 0.10 0.09 0.26 0.74 2.36 11.5 53.16	0 -0.14% -0.14% +171% +100% +571% +900% +1257% +2671%	0.07 0.06 0.06 0.19 0.14 0.47 0.70 0.95 1.94	0.10	0.50	Narasimhan et al. 1993
B6C3F1 female mice	single ip injection	24 hr. post dosing relative mRNA units Cyp1A2	0 ug/kg 0.005 0.010 0.025 0.05 0.10 0.50 1.00 5.00	0 0.11 0.10 0.09 0.26 0.74 2.36 11.5 53.16	0 -0.14 -0.28 -0.28 -0.28 -157 +57 +243 +343	0.07 0.06 0.05 0.05 0.05 0.03 0.11 0.24 0.31	0.5	1.0	Narasimhan et al., 1993 1992
B6C3F1 female mice	single ip injection	24 hr post dosing total nuclear AH receptor binding	0 ug/kg 0.005 0.010 0.025 0.05 0.10 0.50 1.00 5.00	0 0.11 0.10 0.09 0.26 0.74 2.36 11.5 53.16		N/A 4.3 7.2 6.2 8.2 6.5 10.6 45.4 111.3			Narasimhan et al., 1993

TABLE XIV DOSE - RESPONSE B6C3F1 Female Mice

Liver EROD, Skin EROD, Lung EROD, Hepatic 1a2 Activity

Species	Dosing Regimen	Effect	Administered Dose	Response		NOAEL	LOAEL	Reference
B6C3F1 female mice	repeat dose 5 X per week 13 weeks (65 doses) oral	increase hepatic EROD nm/mg protein/min	0 ug/kg/day (0 ug/kg total) 0.0015 (0.0975 ug/kg) 0.0045 (0.292 ug/kg) 0.015 (0.975 ug/kg) 0.045 (2.925 ug/kg) 0.150 (9.75 ug/kg)	100% 220% 260% 610% 1780% 3965%	126.25 mean EROD 271.4 323.4 763.6 2245.25 4996.0			DeVito et al.1992
B6C3F1 female mice	repeat dose 5 X per week 13 weeks (65 doses) oral	increase skin EROD nm/mg/protein/ min	0 ug/kg/day (0 ug/kg total) 0.0015 (0.0975 ug/kg) 0.0045 (0.292 ug/kg) 0.015 (0.975 ug/kg) 0.045 (2.925 ug/kg) 0.150 (9.75 ug/kg)	100% 170% 280% 1040% 1760% 5280%	0.89 mean EROD act. 1.54 2.5 9.29 15.65 47.0			DeVito et al., 1992
B6C3F1 female mice	repeat dose 5 X per week 13 weeks (65 doses) oral	increase lung EROD nm/mg/protein/ min	0 ug/kg/day (0 ug/kg total) 0.0015 (0.0975 ug/kg) 0.0045 (0.292 ug/kg) 0.015 (0.975 ug/kg) 0.045 (2.925 ug/kg) 0.150 (9.75 ug/kg)	100% 217% 545% 1614% 2833% 8786%	4.2 9.15 22.9 67.8 119 369			DeVito et al., 1992
B6C3F1 female mice	repeat dose 5 X per week 13 weeks (65 doses) oral	increase hepatic 1a2 acetanilide 4-hydroxylase	0 ug/kg/day (0 ug/kg total) 0.0015 (0.0975 ug/kg) 0.0045 (0.292 ug/kg) 0.015 (0.975 ug/kg) 0.045 (2.925 ug/kg) 0.150 (9.75 ug/kg)	100% 130% 180% 290% 360% 420%	277.0 357.2 618.4 798.4 920.6 1164.4			DeVito et al.,1992

The fit to the linear model is also poor, $R^2 = 0.54$. The Power Law for P450IA2 induction resulted in a "g" = 0.24, $R^2 = 0.73$, a sublinear functional fit. In additional, the absolute number for this exponent is lower than the "g" value for hepatic, skin or lung EROD induction. The evaluation of data subsets reveals a completely opposite pattern than that observed with EROD, a trend toward a decrease in the kinetic order with increasing dose. Because of the variability of the data and low R^2 the significance of these observations can not be determined.

Table XV Power Law Functional Fit
Selected Subsets of Doses from Devito et al. 1992

Dose ng/kg/day	Hepatic EROD		Lung EROD		Skin EROD		Hepatic 1A2	
1.5 - 15	.47	.69	.87	.92	.75	.80	.34	.59
1.5 - 45	.85	.63	.78	.94	.71	.85	.27	.65
1.5 - 150	.68	.92	.79	.96	.75	.93	.24	.73
4.5 -150	.2	.96	.77	.95	.81	.91	.11	.53
15 - 150	.82	.94	.74	.91	.74	.81	.18	.36

Human and animal P450 enzymes differ both quantitatively and qualitatively. Understanding the role of these enzymes in the multistep process of TCDD induced carcinogenesis is essential if enzymes are to be used as response surrogates. Additionally, the role of these enzymes in immunotoxicity, male reproductive toxicity, teratogenicity, or other non-cancer endpoints is not understood and is an area where additional research is needed.

The role of CypIA1 in the toxicity of TCDD is not yet fully understood. CypIA1 is constituitively expressed both hepatically and extrahepatically in rodents. In man CypIA1 is present and inducible extrahepatically, and current investigations in its role in lung cancer indicate that genetic variations of the CYPIA1 gene may increase the relative risk of lung cancer in humans.

Induction of CypIA1 by TCDD in the liver has been used to evaluate cancer risk. Evidence that rodents and man may induce this enzyme differently may indicate that the estimation of human health risks based on rodent CypIA1 induction is not appropriate. CypIA2 is present constitutively in human livers at widely variable levels, but is not found extrahepatically in humans and in rodents (Wrighton and Stevens 1992). In rodents CypIA2 is found constituitively at higher levels that rodent CypIA1 (Trischer et al. 1992; Kedderis et al. 1991) The induction of CypIA2 may alter the rate of metabolism of endogenous materials thereby altering their fuction. If enzyme induction is used as a surrogate for TCDD toxicity then extrahepatic induction of CypIA1 and hepatic induction of CypIA2 are areas of investigation relevant to human health effects.

Sunahara et al. (1989) reported a dose-dependent decrease in hepatic EGF receptor after administration of 1.0-25.0 ug TCDD/kg po in Sprague-Dawley female rats (Table XVI). EGF is not produced in the liver, but extrahepatically, therefore the levels of EGF in adrenalectomized S-D rats was also investigated. A decrease in the EGF receptor binding was found from 1.0 to 5.0 ug TCDD/kg. Graph Q depicts similiar hepatic EGF receptor binding in intact and adrenalectomized S-D rats administered a dose range of 1 to 5 ug TCDD/kg. However, at doses below 1.0 ug TCDD/kg in adrenalectomized animals there was a marked increase in EGF receptor binding levels. Unfortunately, doses between 0.001 and 0.5 ug TCDD/kg were not tested in intact rats.

An 80-90% decrease in high and low affinity hepatic EGF binding was observed in C57BL/6J mice 7 days after treatment with 30 ug TCDD/kg. There was no decrease in EGF receptor mRNA as a consequence of TCDD treatment indicating that the effect may ocur post-

transcriptionally. The transcriptional alteration of an EGF receptor ligand has been a hypothesized effect of TCDD (Lin et al. 1991). The significance of these decreases to carcinogenesis was discussed in this document (Lucier Chapter). EGF may be involved in the carcinogenic mechanism, but there are not clear cut dose-response relationships in the path from decreased hepatic EGF to cancer.

Epidermal growth factor is believed to be a regulator of cell proliferation (Clarke et al., 1991). A dose-dependent increase in EGFr detected immunohistochemically in mouse embryonic uteric epithelial cells (GD14) of C57BL/6N mice was observed (Abbott and Birnbaum 1990a). A consequence of this is hyperplasia of the uteric epithelial cells, and hydronephrosis. There was not enough data points available for this endpoint to fit it to a dose-response curve.

The level of EGR receptor in embryonic palate closure has been studied in C57BL/6n mice (Abbott and Birnbaum 1989) and in humans (Abbott and Birnbaum 1991). Although statistically significant changes in EGF receptor level were not found in the medial epithelial cells of human palate after exposure to TCDD the C57BL/6N mice the EGF receptor level was altered. In normal the normal palate, closure involves the decrease in
EGF receptor and a subsequent programmed cell death, or lack of cell growth. A sufficient decrease in EGF receptor does not occur in the TCDD treated palate cultures preventing normal palate closure. It has been hypothesized that other factors (TGF-a, or TGF-b play a role in its regulation, but sufficient data is not available to determine a dose-response relationship (Abbott et al. 1991)

TABLE XVI DOSE-RESPONSE FEMALE SPRAGUE-DAWLEY RATS

Epidermal Growth Factor Response Intact V. Adrenalectomized rats

Species	Dosing regimen	Effect	Dose	Response % Change	NOAEL	LOAEL	Reference
Female Sprague-Dawley rats	single p.o	% change EGF receptor binding levels 10 days post-dosing	0 ug/kg 1.0 2.5 5.0 10.0 25.0	0 -14% -19% -39% -54% -64%	no significance determined	no significance determined	Sunuhara et al., 1989
Female Sprague-Dawley rats (200-250 g) adrenal-ectomized.	single p.o	% change EGF receptor binding levels 10 days post dosing	0 ug/kg 0.001 0.01 0.10 0.50 1.0 2.5 5.0	 -3% +27% +2% +7% -12% -48% -43%	no significance determined	no significance determined	Sunuhara et al., 1989

III. CONCLUSION

Understanding the consistencies and inconsistencies of dose response relationships is necessary and can add confidence to a risk assessment. TCDD has been extensively studies with various species and strains of animals using different protocols resulting in many consistencies in response. Thymic atrophy, wasting syndrome and liver toxicity are seen in most controlled animal experiments with TCDD. TCDD is also acutely toxic in many species tested, but the sensitivity to acute toxicity differs with species and strain and does not appear as a constant predictor of other endpoints. Female rats and guinea pigs are less sensitive to acute toxicity than males, but are more sensitive to chronic toxicity to liver. Hamsters are relatively insensitive to acute toxicity but are the very sensitive to fetal toxicity (hydronephrosis). Carcinogenic responses are less well understood but TCDD is clearly a low-dose rodent carcinogen. Where humans respond to each of these endpoints is a critical question in risk assessment which can not be easily answered without a better understanding of the underlying mechanisms of toxicity.

The segregation of toxic effects with the Ah receptor is an often reported phenomenon and it is clear that most responses to TCDD in cells in culture and in animal tissues segregate with the Ah receptor. Generally atypical tissue responses within species are also Ah receptor mediated, for example, the response of BALB/cByJ mice (Ah^b/Ah^b) to TCDD in the PFC assay for immunoresponse is very sensitive, whereas the DBA mouse (Ah^d/Ah^d) is much less sensitive (Silkworth et al., 1989). The kinetics of tissue distribution appear to have some role in different sensitivities. For instance, the half-life in the thymus of the hamster is short and this species requires a comparatively high dose of TCDD to produce thymic atrophy. Pohjanvirta et al., (1989, 1990) reported the same phenomenon in Long Evans and Han Wistar rats, finding that the half-life

in the thymus was related to the animals sensitivity to thymic atrophy. These differences can be accounted for by several factors; primarily pharmacokinetic and dynamic differences. However, as with steroids, tissue and cell factors may also be involved to account for specificity.

CypIA1 or IA2 induction have served as the major surrogates to TCDD activity and has led to a better understanding of tissue susceptibility and species differences. Induction of CypIA1 in humans is difficult to quantify however, CypIA2 has been proposed as a reasonable biomarker when coupled with tissue levels of TCDD (or congeners). The level of CypIA2 in the human liver (which has highly variable constitutive levels) may account for differences in human responses. The role of CypIA1 and CypIA2 induction in cancer is described in this document(Lucier chapter). The relationships between enzyme induction and the increase in toxicity at non-cancer endpoints is not well understood, and this is an area where further research is needed.

In animal studies graphic analysis of the data revealed that the Power Law function was the best fit for dose-response relationships of Total Ah receptor binding, CypIA1 mRNA concentrations and EROD activity. The consistency of the power function seen with the graphic analysis of these endpoints (Graph K) indicates that there is little post-translational modification. The "goodness of fit" to the power function was high for these responses and lines were parallel, confirming what is known of the biological relationships between receptor, mRNA and protein. Graph M shows excellent correlation coefficients for the EROD activity in three tissues, revealing a good fit (R^2 >0.90) to the Power Law function. The consistency of the Power Law function demonstrates that EROD activity in extrahepatic tissues is probably not modified, and from this, indicates that in mice liver EROD activity is a good surrogate for extra hepatic tissue activity. The difference in the power law for hepatic CypIA2 confirms what is known about CypIA2; that is, it regulated

differently that CypIA1. Finally, the analysis of selected data sets with the Power Law function has added to the understanding of the dose-response relationships for these endpoints. The enzyme endpoints are easily analyzed and may prove to be a simple surrogate for some other toxic effects. An "inertial" response in the low dose range can be characterized when applying this function to total P450, EROD and benzo(a)pyrene hydroxylase in the liver. This suggests that the response at low doses may be part of an adaptive response to environmental levels of TCDD ("external forces") and that only at higher doses does the system respond with an increase in kinetic order. The curve fitting for the CypIA1 /CypIA2 regulated endpoints is contrasted to the immunotoxicity data, where the measured endpoints are reached, presumably, through a more complex and less well understood pathway.

Immunotoxicity and thymic atrophy are frequently reported toxic responses to TCDD administration. The application of the Power Law function to these endpoints suggests a very different response than that seen in enzyme induction. At low doses a high kinetic order (reaction rate) is seen contrasted with a lower kinetic order at high doses. This suggests that additional factors, not directly related to enzyme induction, are involved in the these clinical endpoints. Although the immune system may be extremely sensitive to the toxic effects of TCDD there does not seem to be an assay that has developed the sophistication to make interspecies comparisons.

Some organ weight changes were sensitive clinical endpoints. Thymic atrophy and liver weight increases were observed after a single oral dose of 1 ug TCDD/kg in mice, and with a continuous dose of 4.9 TCDD ng/kg/day in guinea pigs (See Table III and Table XI). Although individual data sets fit the Power Law function with a high correlation coefficient, there wasn't enough consistency between study methodology to adequately compare studies. Current research

in the cellular mechanism of thymic atrophy and understanding of thymocyte maturation has developed to the point where a mechanistic model can be proposed. Additional quantitative data on thymocyte maturation across species is needed for this model to develop.

The development of a in vitro model for cleft palate has enabled direct species comparison, and has been useful in understanding these species differences. Qualitatively the measured endpoints in this model have shown consistency across species and have helped to understand the role of the EGF receptor in programmed cell death. There were not enough data points to analyze this endpoint using the Power Law function. Although, qualitative comparisons indicate that rat and human palate are less sensitive than the palates of C57BL/6N mouse. Because of the understanding of the mechanism for cleft palate formation a mechanistic model can be developed.

From a public health perspective, the use of non-cancer endpoints may be more significant in assessing human health risks because some of these effects occur at lower doses and have been reported to occur under general population exposure conditions, ie. short to moderate exposure periods, or acute accidental exposures. A comparison of tissue-dose with effect in a variety of studies exemplifies this (Table XVII). The apparent differences between endpoints for cancer and non-cancer risks are in the extrapolation procedure which are questioned in the modelling chapter (Lucier and Gallo).

Kociba et al. (1978) performed a rodent bioassay to assess the carcinogenic risk of TCDD study supplying Sprague-Dawley rats with feed containing concentrations equivalent to 0, 0.001, 0.01, and 0.1 ug TCDD/kg/day. The administered dose associated with a statistically significant increase in hepatocellular carcinoma in female rats was 0.1 ug TCDD/kg/day. At 0.01 ug

TCDD/kg/day female rats were found to have a statistically significant increase in hepatocellular nodules and alveolar hyperplasia. These doses produced liver concentrations of 24,000 ppt and 5100 ppt, respectively (Kociba 1978).

Using an initiator-promoter dosing regimen, Trischer et al., (1992) found an increase in CypIA1 and Cyp IA2 in rodent liver tissue after administration of weekly doses equivalent to 3.5 ng/kg/day to 125 ng/kg/day. A dose-dependent increase in these biochemical endpoints was significant at all doses. The measured concentration of TCDD in the liver was reported as 480 ppt to 1990 ppt.

In contrast to these two studies, liver concentrations of TCDD (tissue dose) that statistically increases non-cancer endpoints are as low as 0.084 ppt. Narasimhan et al., (1993) administered a single i.p. dose of 0 to 2.5 ug TCDD/kg to B6C3F1 female mice, and found a statistically significant decrease in splenic plaque forming cells (PFCs/spleen) at the 0.05 ug TCDD/kg dose level. The liver concentration that resulted in this decrease was 0.084 ppt.(calculated from Narasimhan et al. 1993)

Ma et al. 1991 administered female C57BL/6J mice single i.p. injections of TCDD and measured the decrease in hepatic protein tyrosine phosphorylation. The EC_{50} for this decreased response was 0.05 ug TCDD/kg which was approximately equivalent to a tissue dose of 0.084 ppt. (the tissue distribution data used to calculate this was from Narasimhan et al., 1993). This data also reports an effect that is not clearly nuclear-Ah receptor mediated, the decrease in protein tyrosine phosphorylation.

Table XVII Summary LOEL Non-Cancer Endpoints vs. Cancer Endpoints See text for description of study protocols		
Endpoint	**LOAEL - Tissue dose - liver ppt**	**Reference**
increase hepatic hyperplastic nodules	5100 ppt	2 year Rodent Bioassay - Kociba et al., 1978
increase Cyp1A1/Cyp1A2	480 ppt	Initiation-Promotion Protocol - Trischer et al., 1992
increase benzo(a)pyrene hydroxylase (Ahh)	5.4 ppt	Kitchin and Woods, 1979
increase EROD	10.2 ppt	Abraham et al., 1988
reduction in spermatogenesis in male offspring	maternal tissue dose 806 ppt fetal liver dose at 0.083 ppt	Mably et al. 1992
Increase in protein tyrosine phosphorylation	0.084 ppt (estimated)	Ma et al., 1992
decrease splenic plaque forming cells	0.084 ppt	Narasimhan et al., 1993

The increase hepatic enzyme activity is a well documented phenomenon which may ultimately be responsible for alterations in clinical endpoints. Kitchin and Woods, 1979 found that enzyme activity (benzo(a)pyrene hydroxylase) was significantly increased in Sprague-Dawley rats after the single oral administration of 0.002 ug TCDD/kg (LOAEL). Based on [1,6-^{3}H] TCDD administration the tissue concentration in the liver that resulted in this increased enzyme activity was 5.4 ppt. Kitchin and Woods (1979) estimated that this level is equivalent to 65 molecules of TCDD per liver cell. Abraham et al. (1988) reported a statistically significant increase in ethoxyresorufin O-deethylase activity (EROD) after a single s.c. administration of 0.003 ug TCDD/kg resulting in a measured ^{14}C-TCDD liver concentration of 10.2 ppt. These two studies are remarkably consistent, in reporting liver concentrations which are less than 1/200 the concentrations in the carcinogenicity bioassays.

Other studies have also found very low TCDD concentrations that result in adverse effects. Mably et al. (1992) reported that a single maternal dose of 0.064 ug TCDD/kg resulted in a significant and permanent reduction in spermatogenesis in their male offspring. If, as VanBerg et al. (1987) reported, that 0.13% of TCDD reaches the fetus, then the fetal dose that resulted in these effects would be 0.083 ppt.

Clearly hepatocellular carcinoma is not the most sensitive effect of TCDD administration in animals. Induction of cancer appears to occur after continuous exposure to doses that result in high levels of enzyme induction. The most significant public health concern from TCDD exposure may be immunotoxicity, reproductive toxicity and other effects that are seen in human and animal after low dose acute exposures. Empirical analysis of these effects will help to determine the biological significance of these observations and result in a more relevant risk assessment.

The evaluation of dose-response relationships has demonstrated many consistencies and inconsistencies in the patterns of toxicity between tissues, strains, and species. The development of new models has been proposed for cleft palate development and thymic atrophy because they are well understood and sufficient data has been collected to analyze additional data needs and start mechanistic model development. Future research focused on cleft palate, thymocyte maturation and immunotoxic endpoints, and male reproductive toxicity should result in progress toward this end.

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Appendix 1

The Use of Power Laws, Michaelis-Menten and Hill's Function in Dose Response: The Mathematical Rationale

The use of a particular empirical function in phenomenological dose-response analysis is guided by conceptual considerations concerning infinitesimal (differential) incremental changes in response at a given dose level. In the following x and y denote dose and response in appropriate units, $y = f(x)$ the dose response function and dx, dy are the differentials of the dose and the response. As a simple example, one may consider the case of linear response: the underlying assumption is that the infinitesimal change in response is always proportional to, and with a constant proportionality ratio, a, to the infinitesimal change in dose at all dose levels. So, $dy = adx$, which is integrated to give $y = ax+b$, b being the "response at zero dose".

A nonlinear dose-response function is derived if one assumes that at any given level of dose and infinitesimal change in response is proportional to an infinitesimal change in dose but with a proportionality ratio that depends on the levels of dose and response. The specific assumption that leads to a Power Law is that the incremental change in response will be proportional to the incremental change in dose as well as to the total response at that point, but inversely proportional to the total level of dose: $dy = g dx \frac{y}{x}$, where g is a a proportionality constant. This relationship gives, $y = ax^g$, where a is an integration constant. The differential relationship above essentially states that the local rate of change of response with increasing dose is proportional to the total response normalized over the total dose. This concept,

i.e. the rate of change of the "dependent" variable with respect to the "independent" variable being proportional to their ratio, underlies much of the S-system approach to nonlinear phenomenon and employs a type of scaling that is common in many physiological and other biological systems. More complicated nonlinear dose-response functions are derived if one assumes a similar but generalized relationship between incremental response and dose locally, i.e. at a given point of the dose-response curve, as follows:

$$dx = g(x)dx\frac{y}{x}$$

where the proportionality constant g is now replaced by a more general "local modifier function" $g(x)$. By assigning an appropriate form to $g(x)$ one can appropriately modify the relationship between dose and response so as to reflect dominance of different biological phenomenon over different ranges of the dose-response curve. For example, one can choose a functional form for g(x) such that for small doses the proportionality factor is close to unity and therefore the relationship $dy = gdx\ \frac{y}{x}$ becomes linear or assumes another constant value, g, in which case the power law behavior (with power g) dominates at low doses. At the same time one may define $g(x)$ so that e.g., at high doses the incremental change in response becomes vanishingly small. A functional form that combines both these requirements is:

In that case

$$g(x) = \frac{gb}{b+x^g}$$

$$\frac{1}{y}\frac{dy}{dx} = \frac{1}{x}\frac{gb}{b+x^g}$$

and, using the property

$$\int \frac{dx}{x(b+x^g)} = \frac{1}{bg} \ln \left| \frac{x^g}{b+x^g} \right|$$

and one gets

$$y = \frac{ax^g}{b+x^g}$$

where a is an integration constant. The above expression is the well known Hill function (which for $g=1$) becomes the Michaelis-Menten equation.

GRAPH A1

Dose-Response

(Graph in Log-Linear Coordinates)

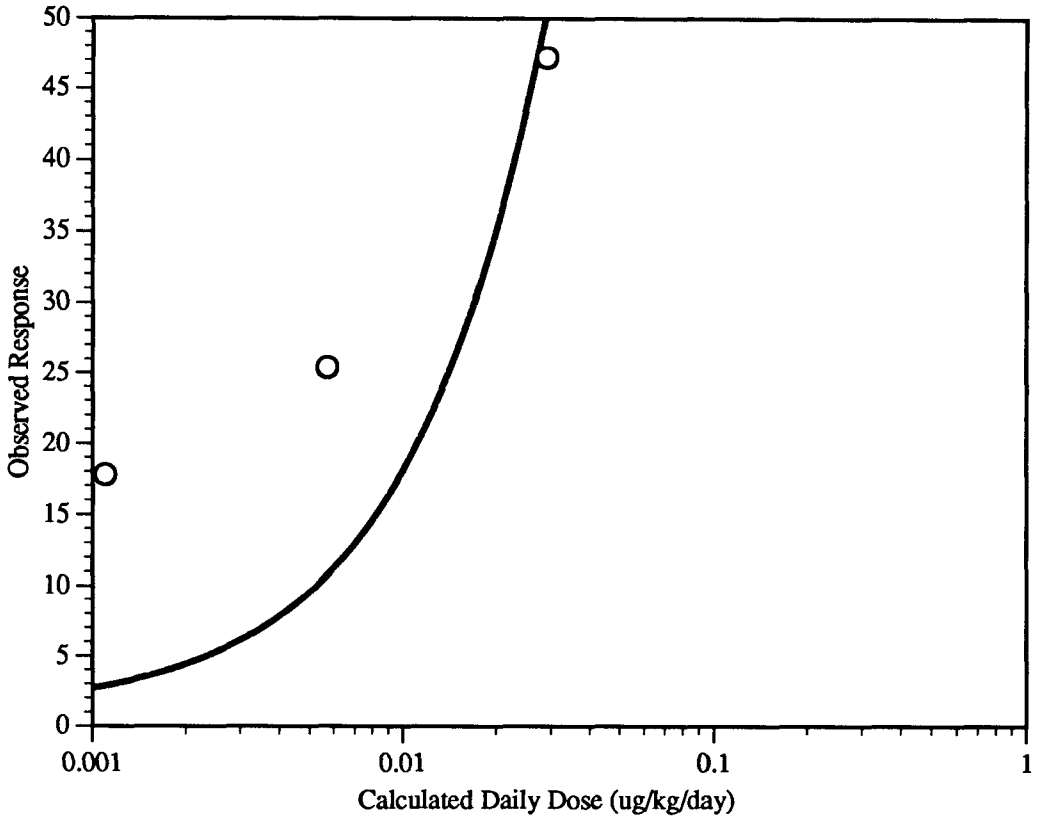
for

Female Guinea Pigs

(Harris et al., 1973)

Dose: repeat oral dose 1 X weekly for 8 weeks

Response: % decrease thymic weight



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1 + Michaelis-Menten" type response)

f(x) =1+4.745175E+6*x/(2.771639E+3+x), chi^2 = 447.7731

GRAPH A2

Dose-Response

(Graph in Log-Linear Coordinates)

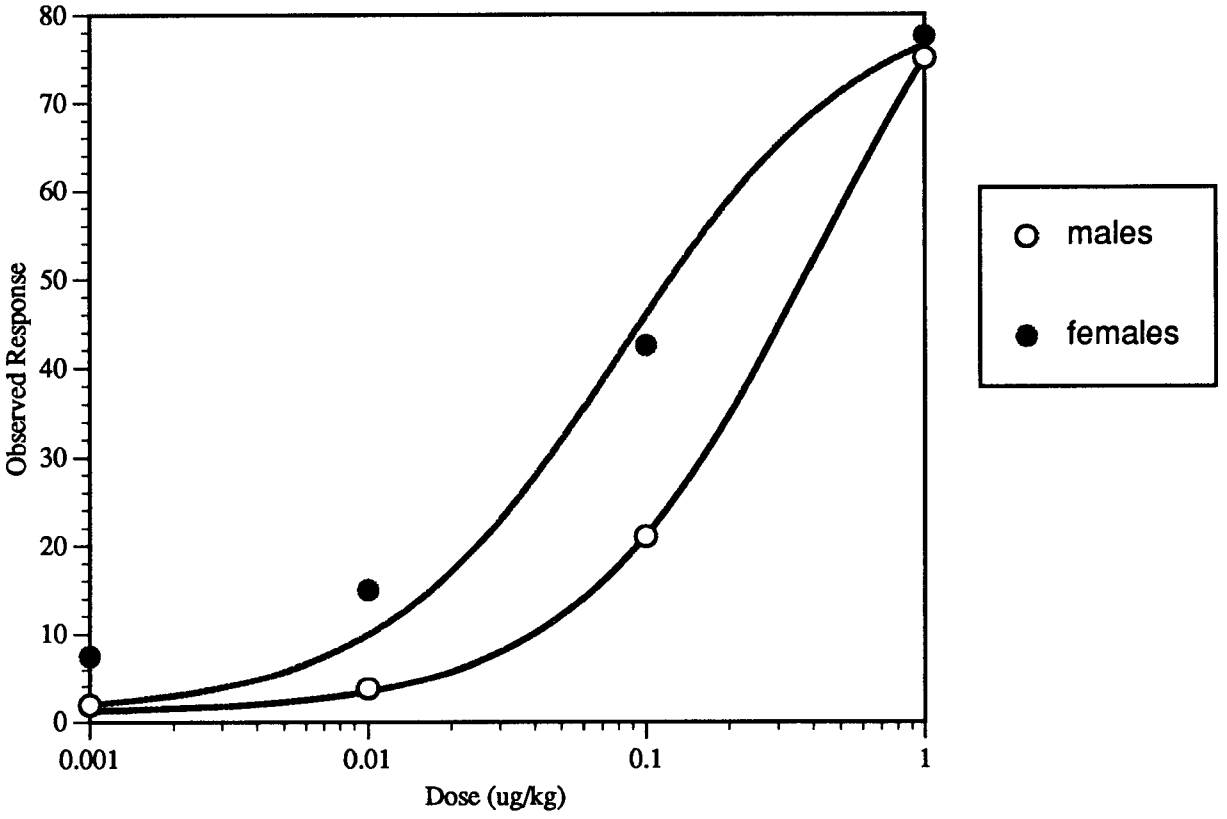
for

Male and Female Sprague-Dawley Rats

(Kociba et al, 1976)

Dose: repeat dose 5 X per week for 13 weeks

Response: % Decrease thymus weight



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1 +Michaelis-Menten" type response)

males, f(x) = 1+1.054825+2*x/(4.255322E-1+x), chi^2 = .5741008

females, f(x) = 1+8.165068E+1*x/(8.180588E-2+x), chi^2= 69.15886

GRAPH B1

Dose-Response

(Graph in Log-Linear Coordinates)

for

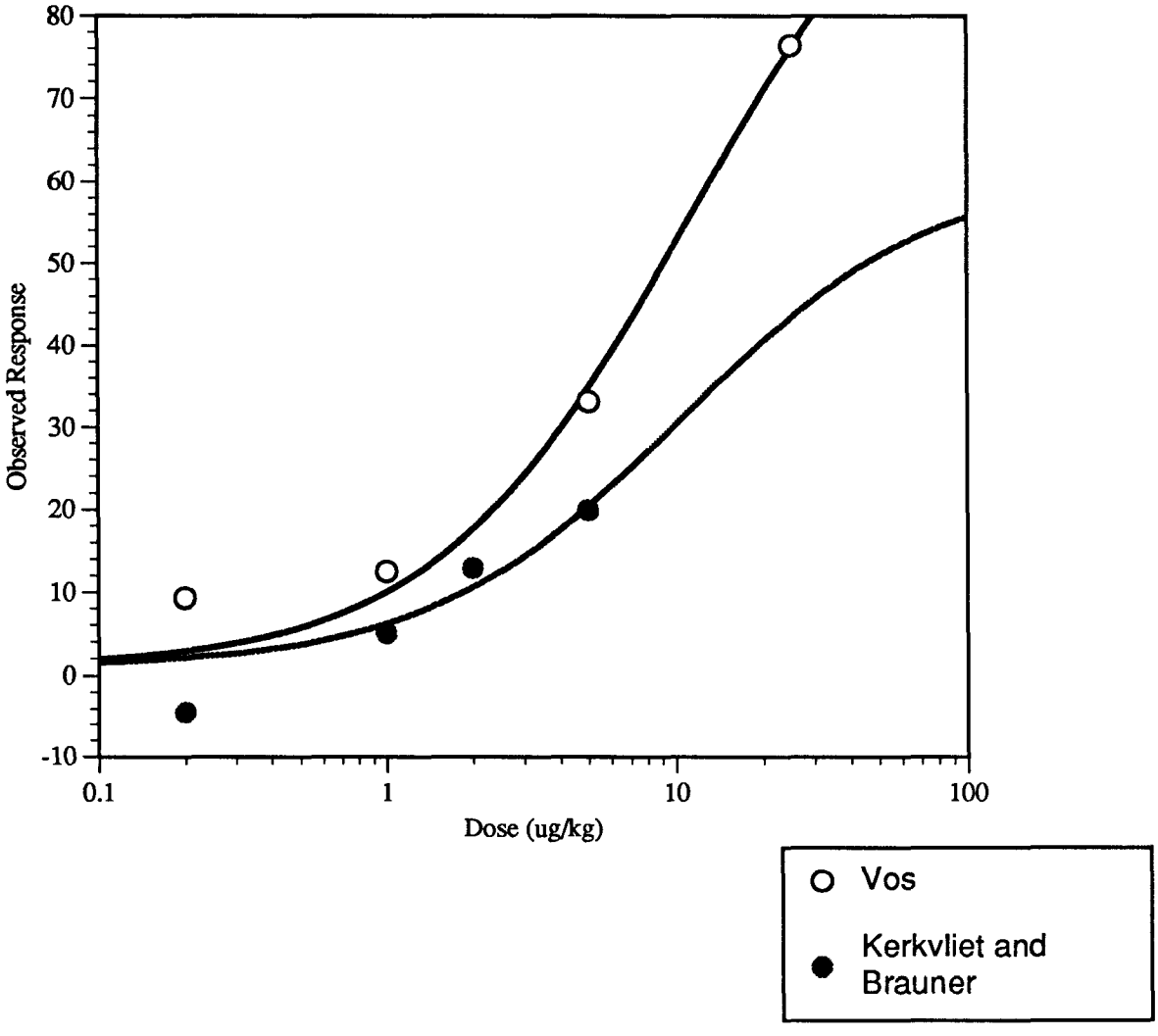
Male C57BL/6 Sch St. mice, (Vos, 1974)

Female C57BL/6 mice (Kerkvliet and Brauner, 1990)

Dose: single oral

Response: % decrease in thymic weight 2 weeks (Vos) and 7 days

(Kerkvliet and Brauner) post dosing



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1 +Michaelis-Menten" type response)

Vos, f(x) = 1+6.046204E+1*x/(1.061148E+1+x), chi^2 = 50.5892

Kerkvliet and Brauner, f(x) = 1+ 1.081043E+2*x/(1.098941E+1+x), chi^2 = 49.71537

GRAPH B2

Dose-Response

(Graph in Log-Linear Coordinates)

for

Male C57BL/6 Sch St. mice, (Vos, 1974)

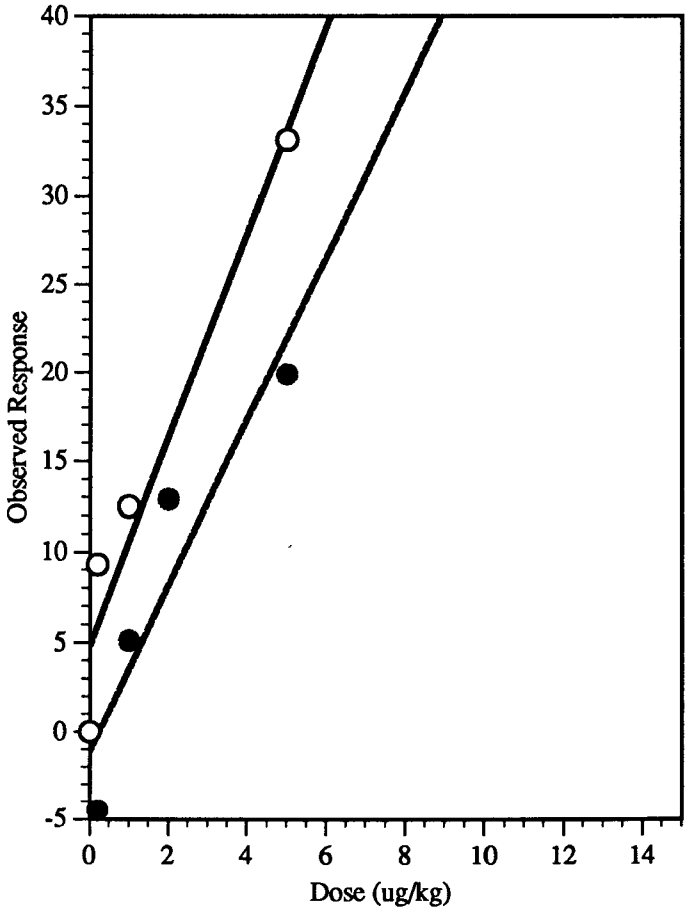
Female C57BL/6 mice (Kerkvliet and Brauner, 1990)

Dose: single oral

Response: % decrease in thymic weight 2 weeks (Vos) and 7 days

(Kerkvliet and Brauner) post dosing

A



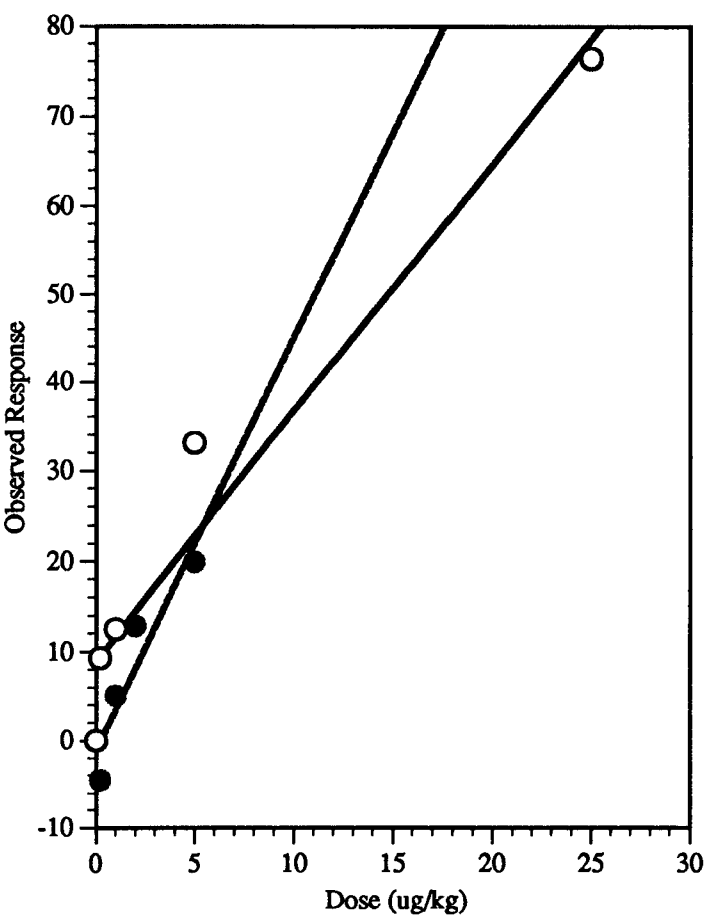
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for Linear type response)

Vos,f(x) =5.767803E+0*x+4.784906E+0, R^2 = .93

Kerkvliet , f(x) = 4.613001E+0*x+-1.106652E+), R^2 = .85

B



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Linear type response)

Vos, f(x) = 2.784140E+0+8.886964E+0, R^2 = .95

Kerkvliet, F(x) = 4.613001E+0*x+-1.106652E+0, R^2 = .85

Graph B3

Dose-Response

(Graph in Log-Linear Coordinates)

for

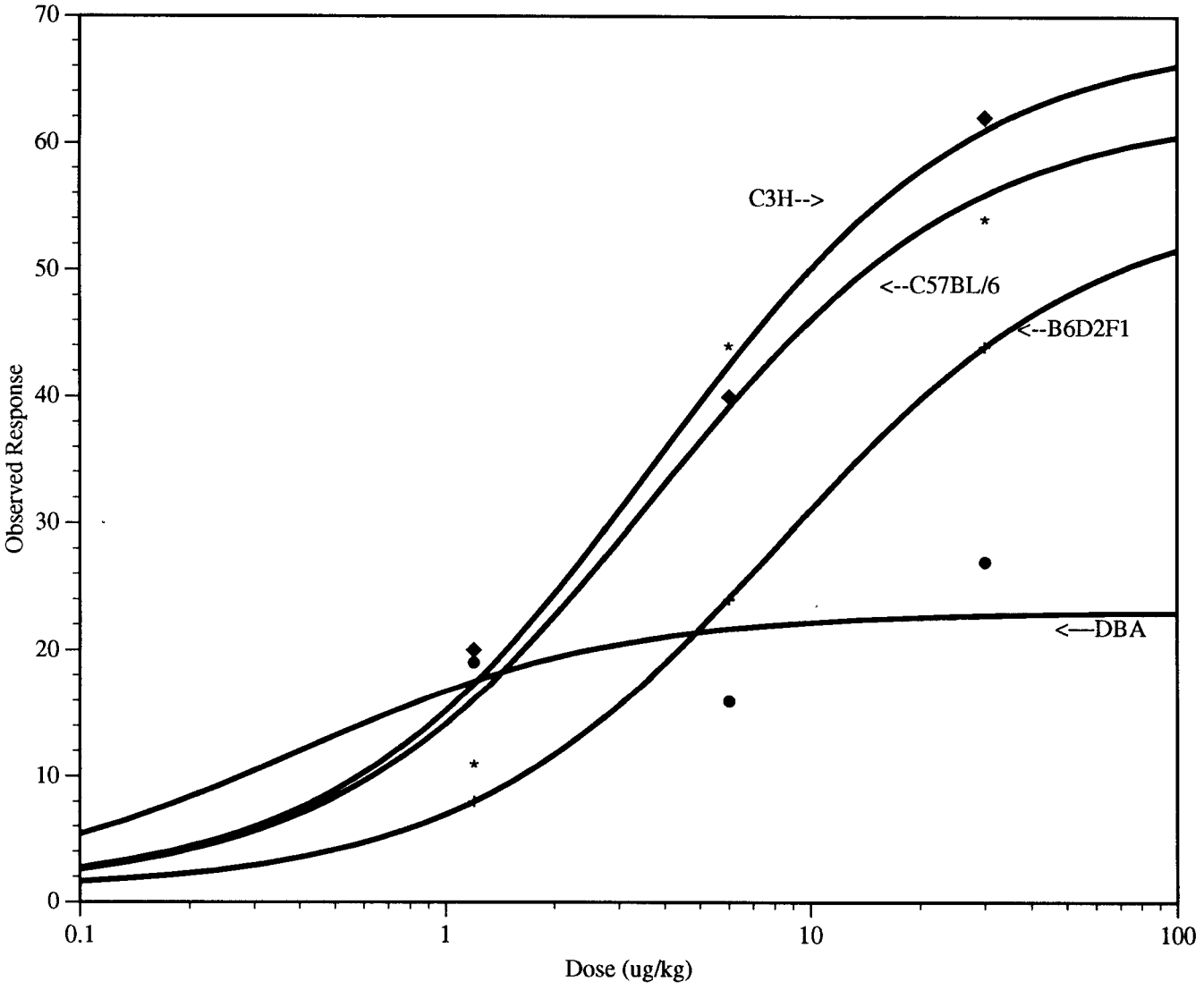
Male mice with differing suseptibility to he Ah induction

(Vecchi et al., 1983)

Dose: single ip

Response: % decrease thymus weight 12 days post-dosing

Michaelis Menten Function



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1+ Michaelis-Menten" type response)

B6D2F1 f(x) = y=1+5.477307E+1*x/(8.229451E+0+x),chi^2 = 0.33

C57BL/6J f(x) = y = 1+6.171059E+1*x/(3.697454E+0+x), chi^2 = 48.9

C3H f(x) = y = 1+ 6.754428E+1*x/(3.767704E+0+x), chi^2 = 14.4

DBA f(x) = y = 2 + 2.20347E+1*x/(3.000336E-1+x), chi^2 =52.3

GRAPH B4

Comparative Dose-Response

(Graph in Log-Log Coordinates)

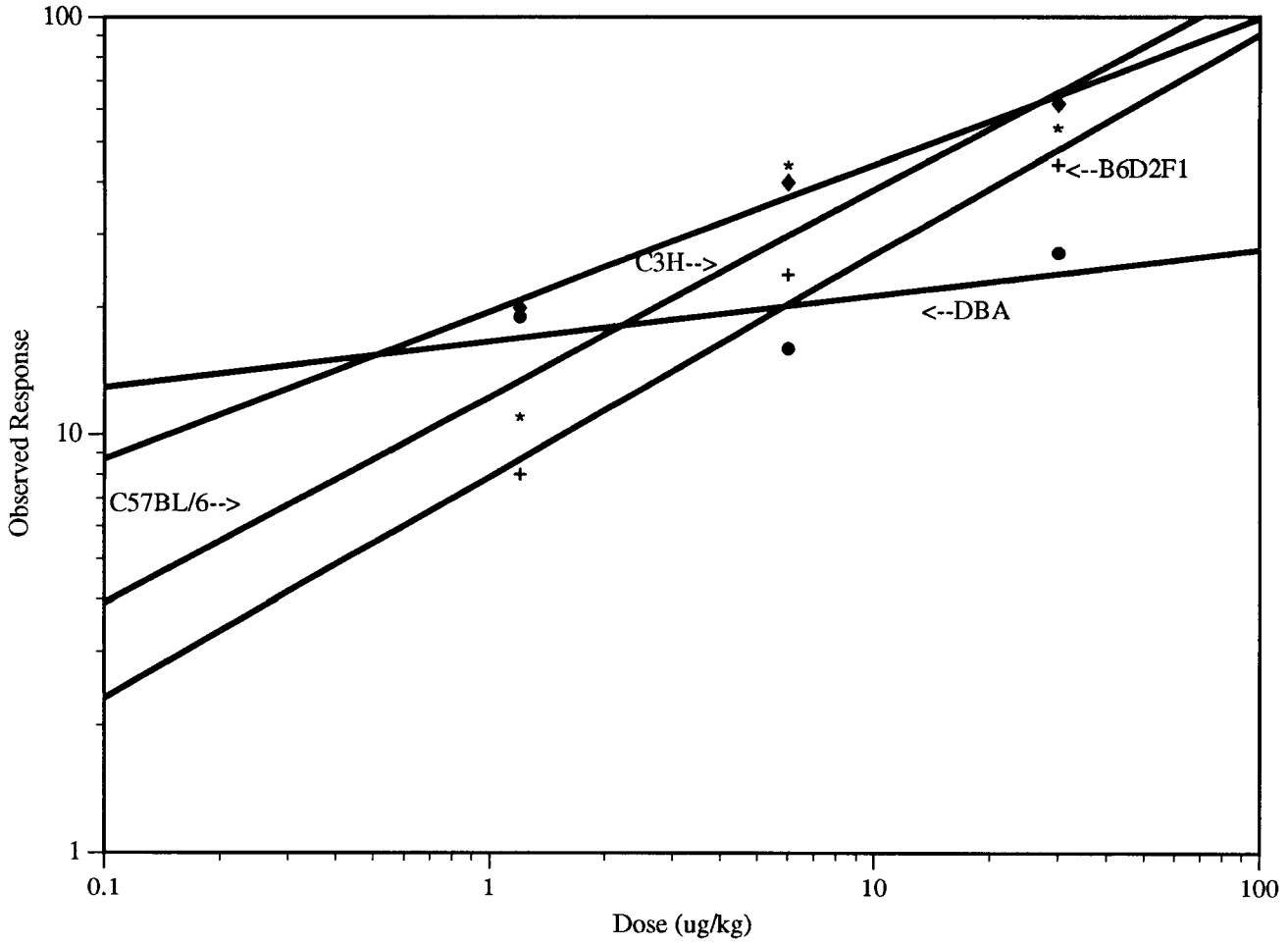
for

Male mice with differing suseptibility to the Ah induction

(Vecchi et al., 1983)

Dose: single ip

Response: decrease thymus weight



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

B6D2: f(x) = 7.884996E+0*(x^5.296098E-1),R^2=9.729347E-1

C57BL/6J: f(x) = 1.223977E+1*(x^4.942995E-1), R^2 = 8.447337E-1

C3H: f(x) = 1.957259E+1*(x^3.514898E-1),R^2 = 9.833631E-1

DBA:f(x) = 1.658810E+1*(x^1.091679E-1), R^2 = 4.339738E-1

GRAPH B5

Dose-Response

(Graph in Log-Linear Coordinates)

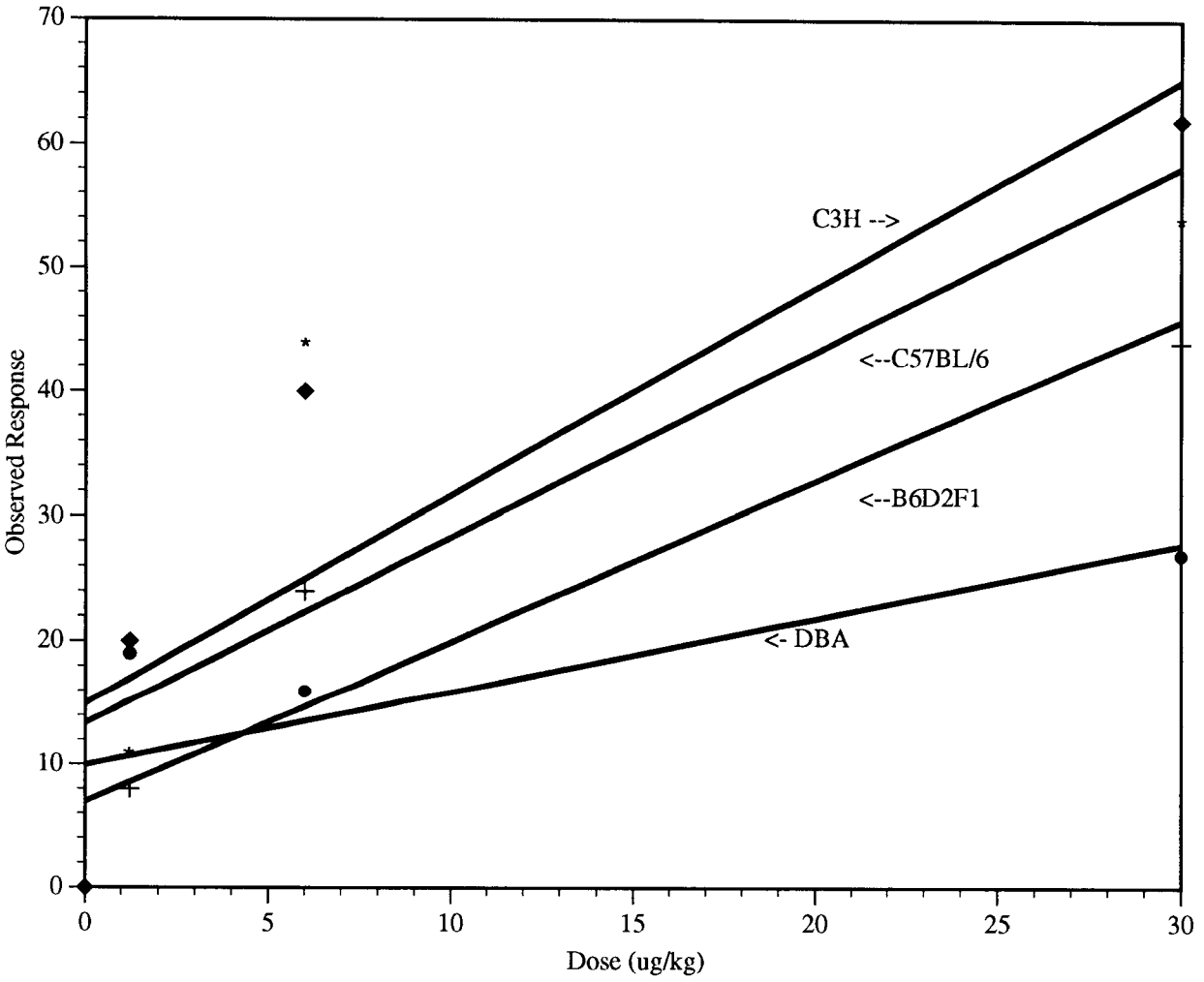
for

Male mice with differing suseptibility to the Ah induction

(Vecchi et al., 1983)

Dose: single ip

Response: % decrease thymus weight 12 days post-dosingDose-Response



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for Linear function)

C57BL/6, f(x) = 1.145833E+0*x +2.212500E+1, R^2 = 6.173469E-1

C3H , f(x) = 1.283602E+0*x +2.475000E+1, R^2 = 8.888266E-1

DBA , f(x) = 3.360215E-1 *x +1.65000E+1, R^2 = 8.313934E-1

B6D2F1, f(x) = 1.115591E+0*x+1.150000E+1, R^2 = 9.107615E-1

GRAPH B6

Dose-Response

for

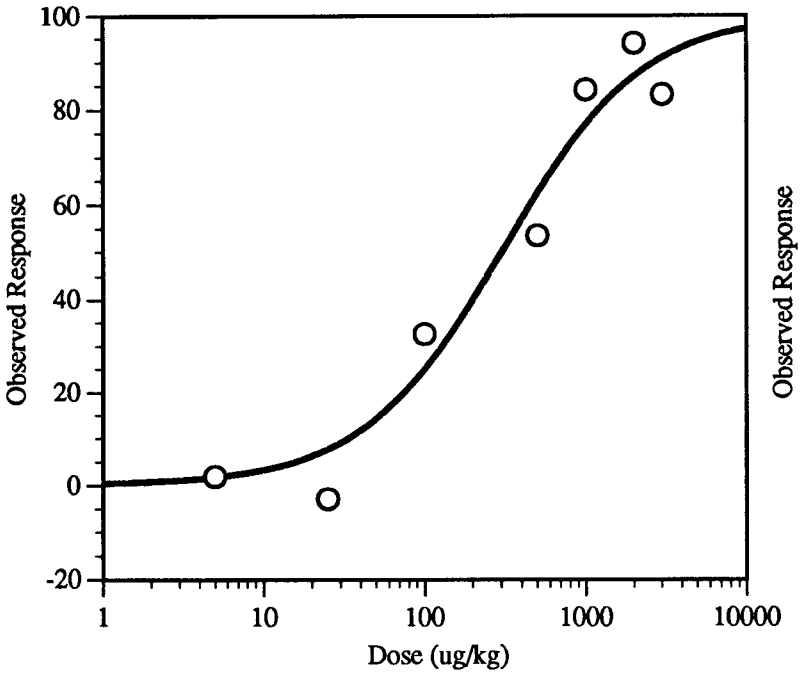
male Syrian Hamsters

Olsen et al, 1980

Dose: single oral

Response: % response decrease thymic weight 50 days post dosing

A



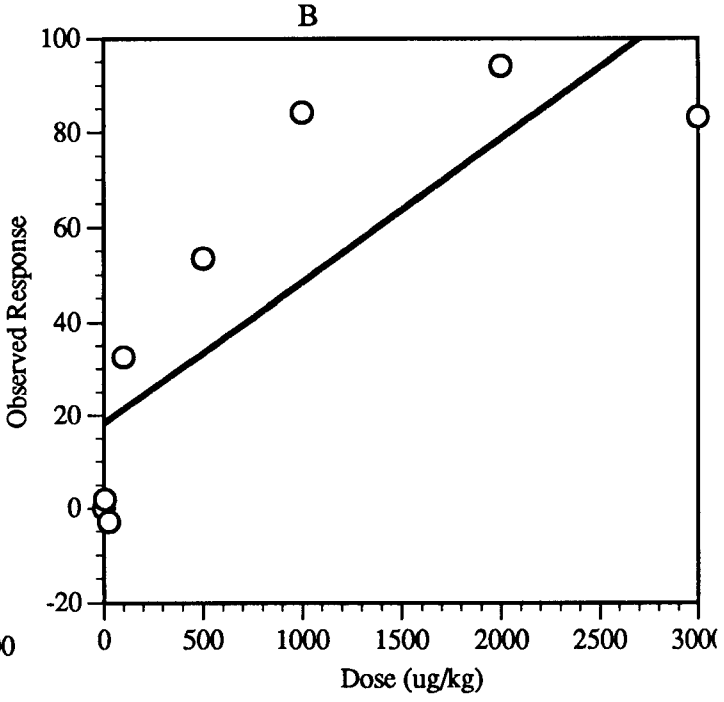
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" type response

f(x) =0.101 + 1.001197E +2 *x/(3.023870E+2+x)

chi^2 = 415.4893

B



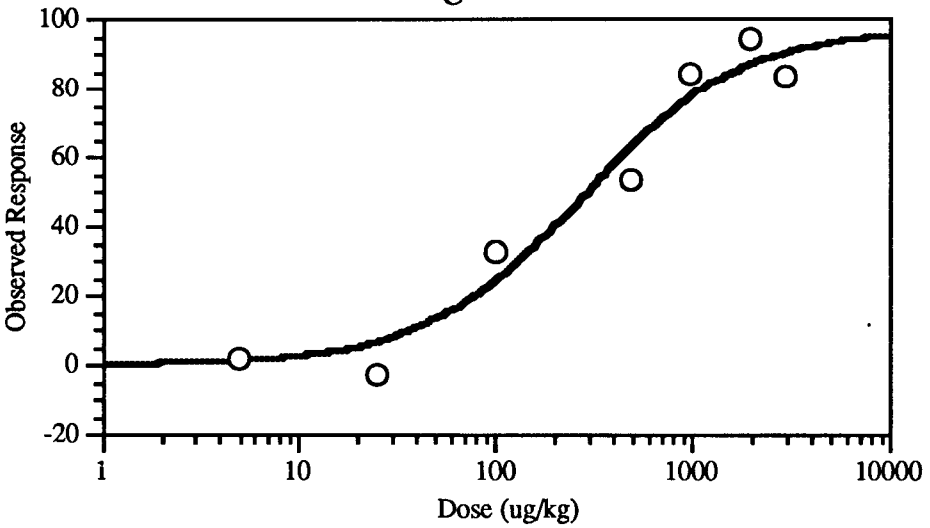
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for Linear type response)

f(x) = 3.019634E-2*x +1.829978E+1

R^2 = 6.781075E-1

C



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill plot)

f(x) = (9.709755E+1 *x^1.087476E+0)/(4.559997E+2+x^1.087476E+0)

chi^2 = 407.9375

Graph B7

Dose-Response

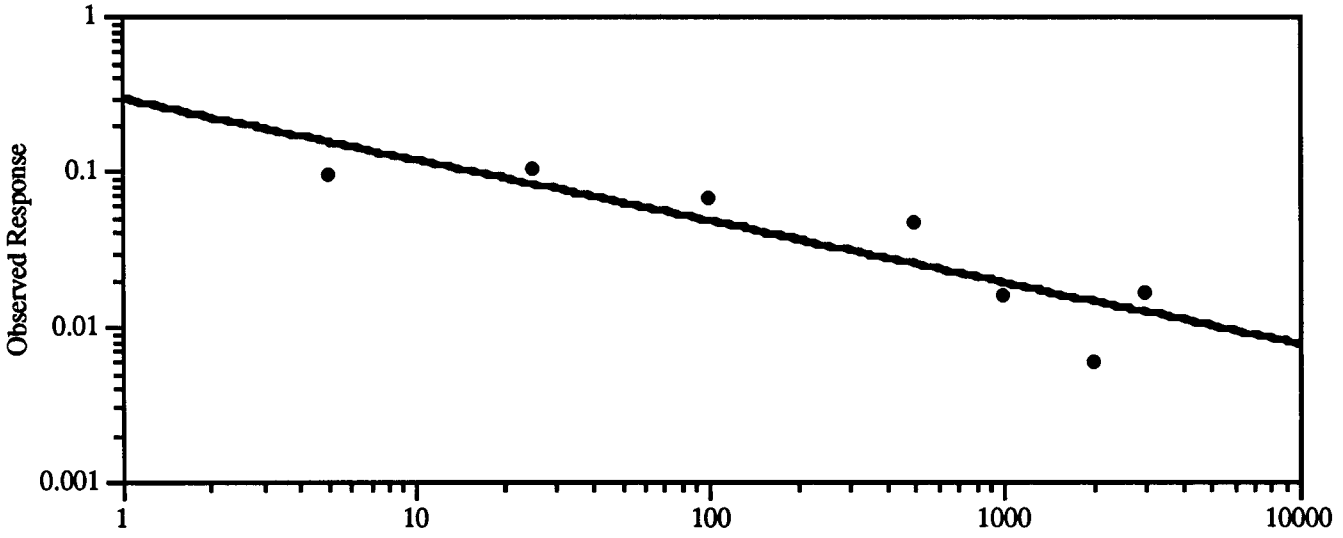
(Graph in Log-Linear Coordinates)

Male Syrian Hamsters

(Olsen, 1980)

Dose: single oral

Response: response thymus weight change after 50 days-(A)- raw data

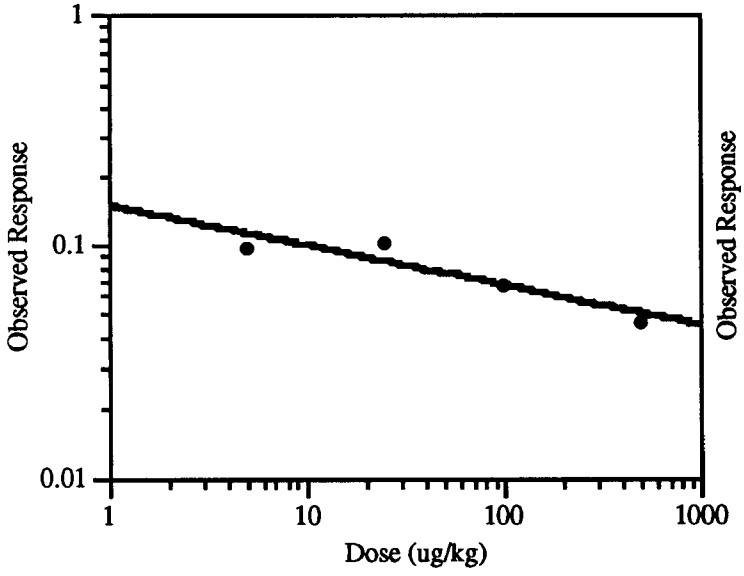


(Best Fit Estimate for Power Law)

f(x) = 2.931550E-1*(x^-3.945743E-1)

R^2 = 7.593673E-1

(B)



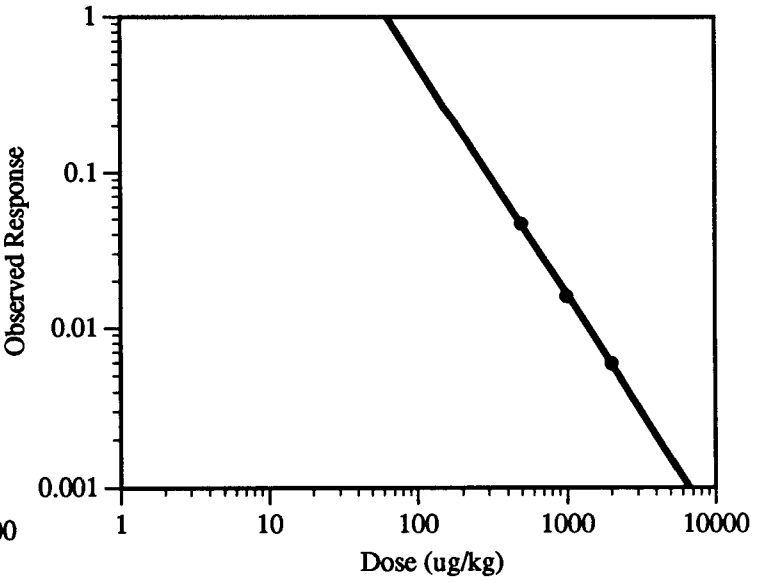
(Best Fit Estimate for Power Law Function)

(doses = 5 - 500 ug/kg)

f(x) =1.494819E-1*(x^-1.737934E-1)

R^2 = 8.487105E-1

(C)



(Best Fit Estimate for "Power Law Function)

(doses 500-2000 ug/kg)

f(x) =4.705032E+2*(x^-1.484813E+0)

R^2 = 9.992644E-1

Graph C1

Dose-Response

(Graph in Log-Linear Coordinates)

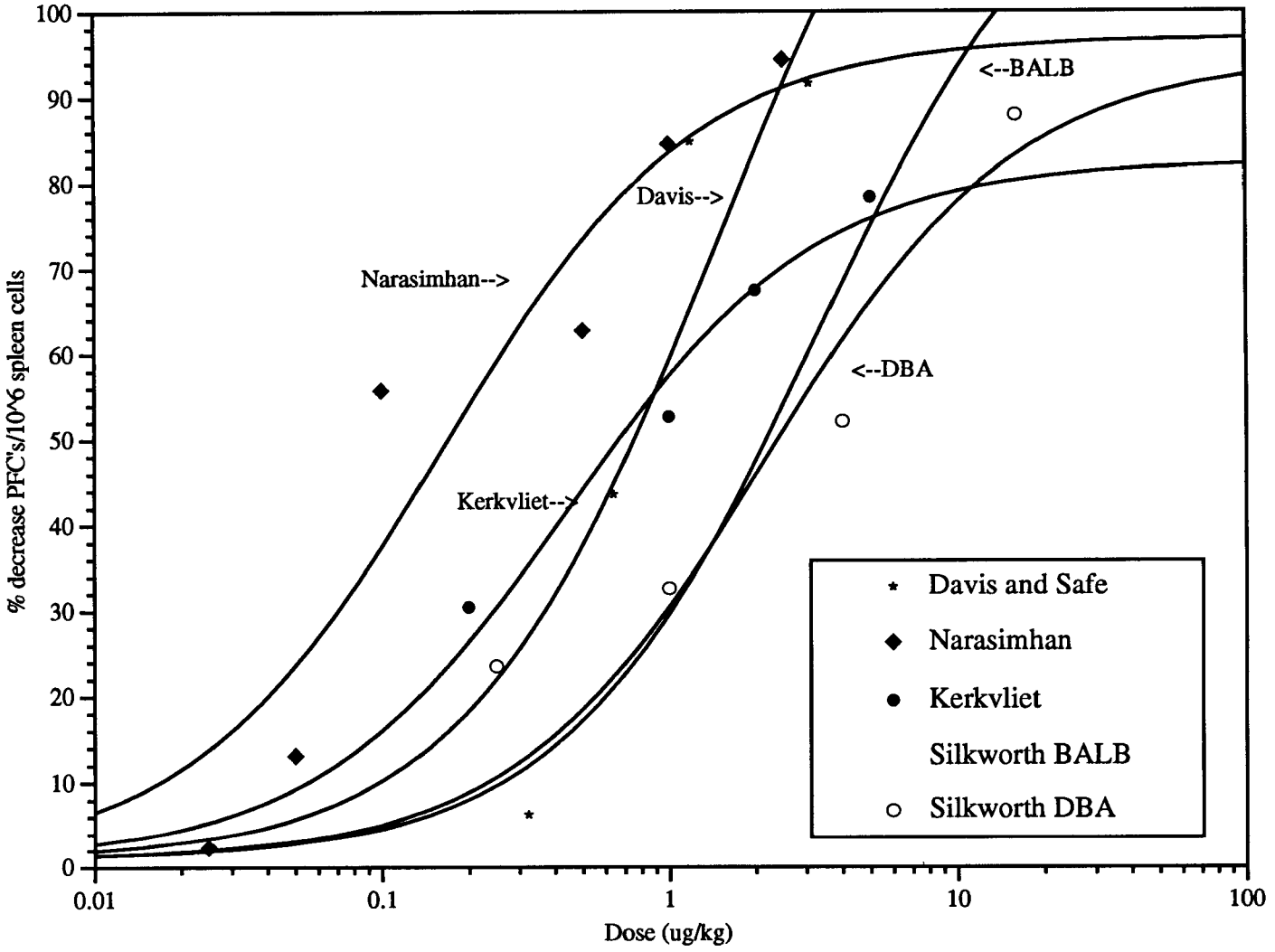
Female C57BL/6 mice (Kerkvliet and Brauner, 1990)

Male C57BL/6J mice (Davis and Safe, 1988)

B6C3F1 female mice (Narasimhan et al.,1993)

BALB and DBA male mice (Silkworth et al., 1989)

Dose: Single oral or ip administration



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1 +Michaelis-Menten" type response)

Davis, f(x) = 1 + 1.432263E+2*x/(1.456438E+0+x), chi^2 =849

Narasimhan et al, f(x) = 1+(9.592833E+1*x/(1.627160E-1+x), chi^2= 693

Kerkvliet, f(x) = 1+ (8.143631E+1*x)/(4.432174E-1+x), chi^2 = 46.2

Silkworth BALB, f(x) = 1+(1.225246E+2+x)/(3.286863E+0+x), chi^2=284

Silkworth DBA, f(x) = 1+(9.344592E+1*x)/(2.176351E+0+x), chi^2=280

Graph C2

Comparative Dose-Response

(Graph in Log-Log Coordinates)

Female C57BL/6 mice (Kerkvliet and Brauner, 1990)

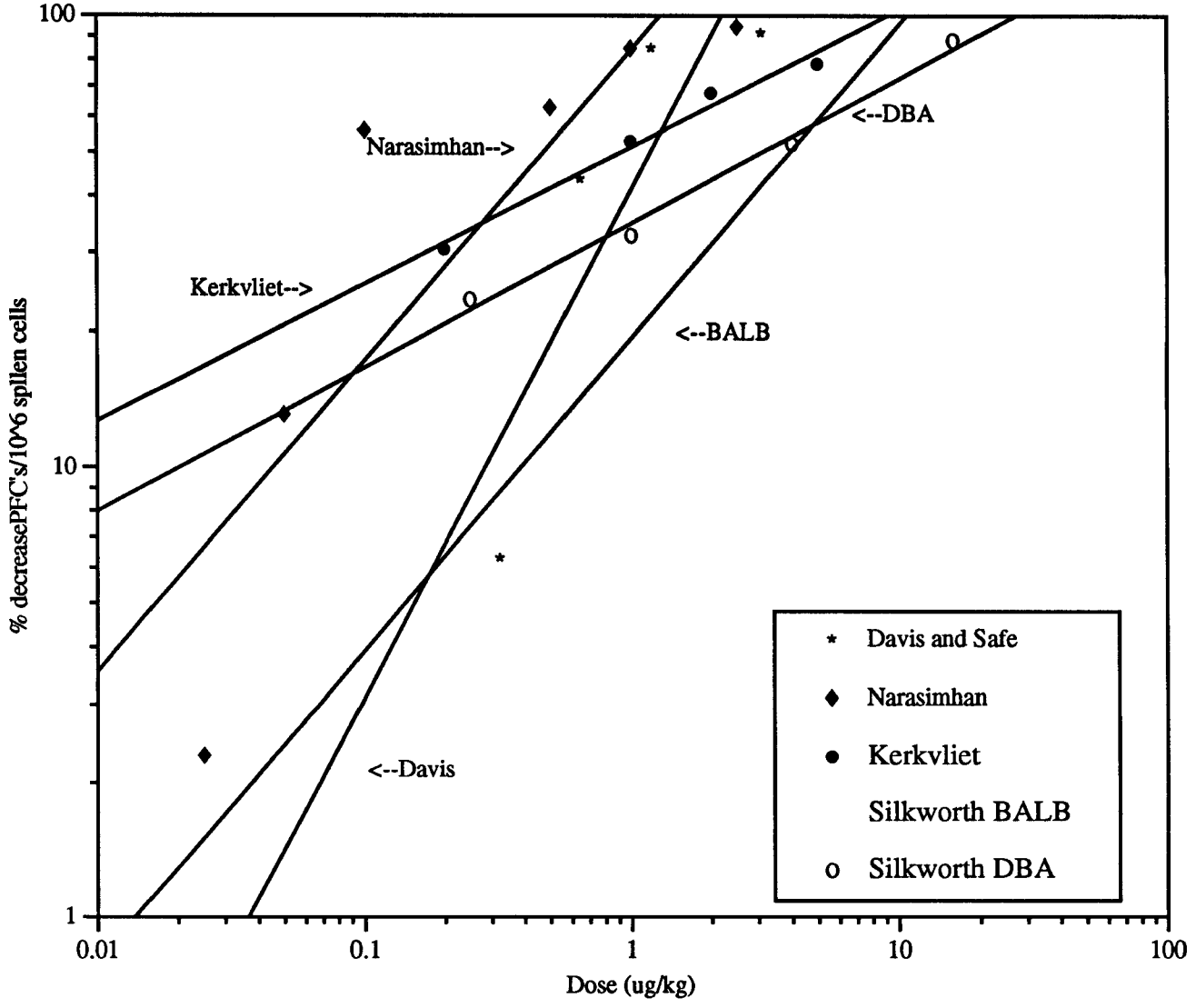
Male C57BL/6J mice (Davis and Safe, 1988)

B6C3F1 female mice (Narasimhan et al., 1993)

BALB and DBA male mice (Silkworth et al., 1989)

Dose: Single oral or ip administration

Response: % decrease PFC/10^6 spleen cells



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

Davis, f(x) = 4.124948E+1*x^1.125711E+0, r^2 = .7501

Narasimhan et al., f(x) = 8.380591E+1(x^6.873447E-1), r^2 = .7317

Kerkvliet, f(x) = 5.114275E+1*x(x^3.027756E-1), r^2= .9807

Silkworth BALB, f(x0 =1.929342E+1 *(x^6.915990E-1), r^2=.9371

Silkworth DBA, f(x) = 3.468637E+1* (x^3.193009E-1), r^2 =.9899

Chart C3

Dose-Response

(Graph in Log-Linear Coordinates)

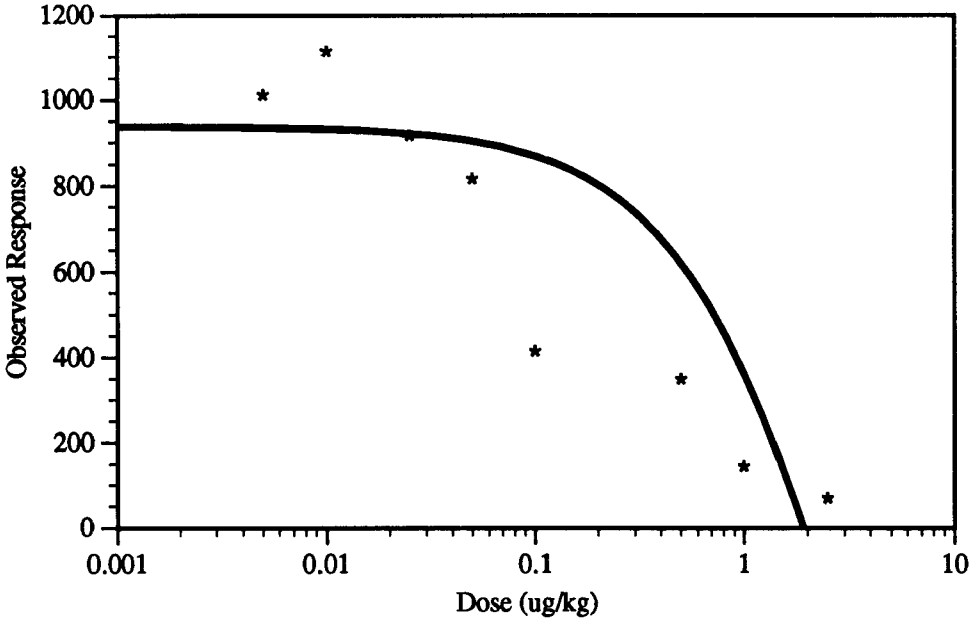
for

Female B6C3F1 mice

(Narasimhan et al., 1993)

Response: number of PFC's/ 10^ 6 spleen cells

A

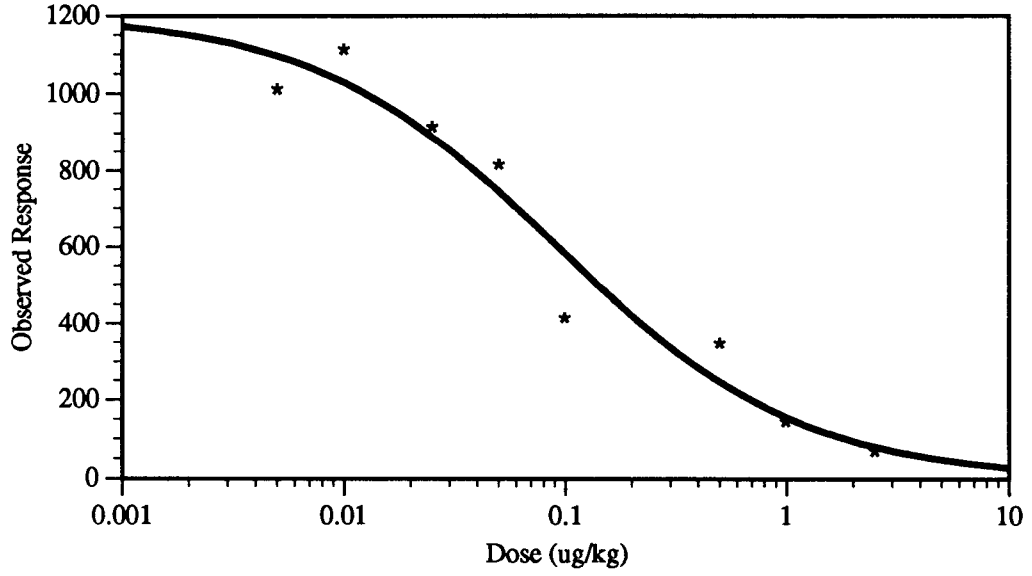


Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" + background parameter type response)

f(x) =939 + -3.005346E+3 *x, chi^2 = 43,447

B



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill function type response)

f(x) = 1.204213E+3*x^-7.995208E-1/6.767036E+0+ x^ -7.995208E-1, chi^2 = 5834

Chart C3

Dose-Response

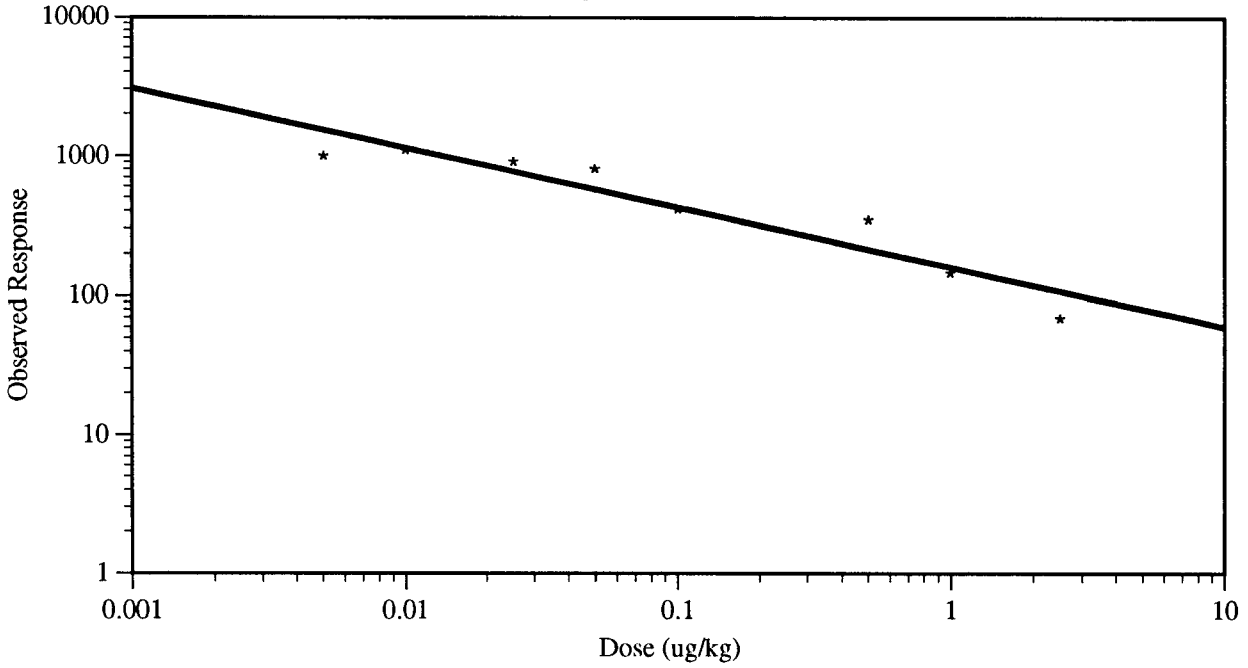
(Graph in Log-Linear Coordinates)

Female B6C3F1 mice

(Safe, 1992)

Response: % decrease PFC

C

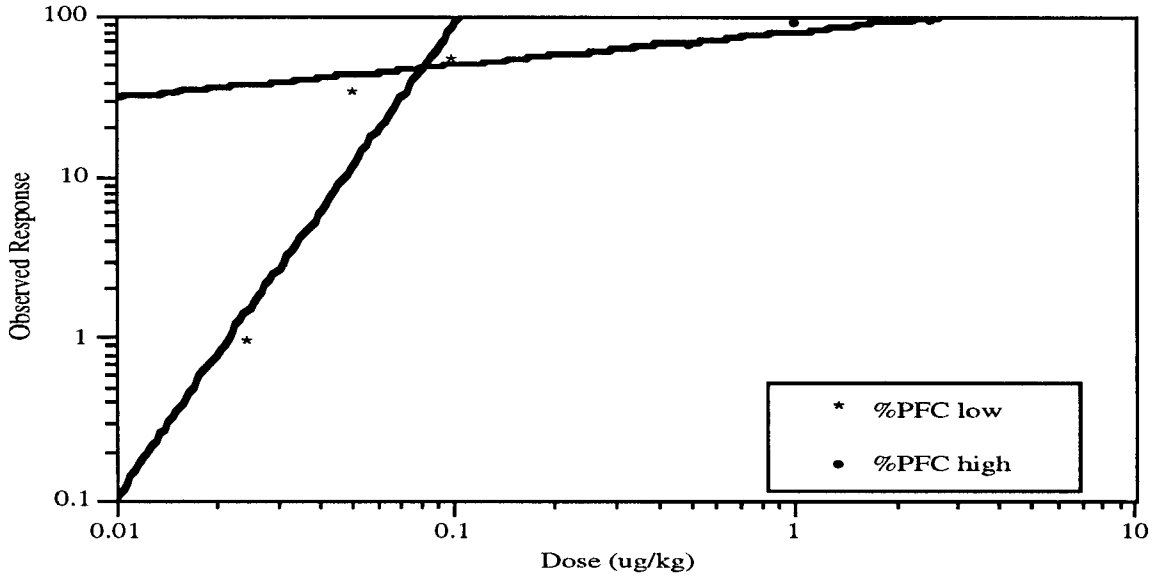


Functional Form of Dose-Response

(Best Fit Estimate for Power Law)

f(x) =1.602198E+2*x^-4.269049E-1, r^2 = .8930

D



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

PFC low dose f(x) = 7.395768E+4*(x^2.933124E+0), R^2=8.481507E-1

PFC high dose f(x) =8.103178E+1*(X^2.074615E-1), R^2 = 8.233572E-1

GRAPH C4

Dose-Response

(Graph in Log-Linear Coordinates)

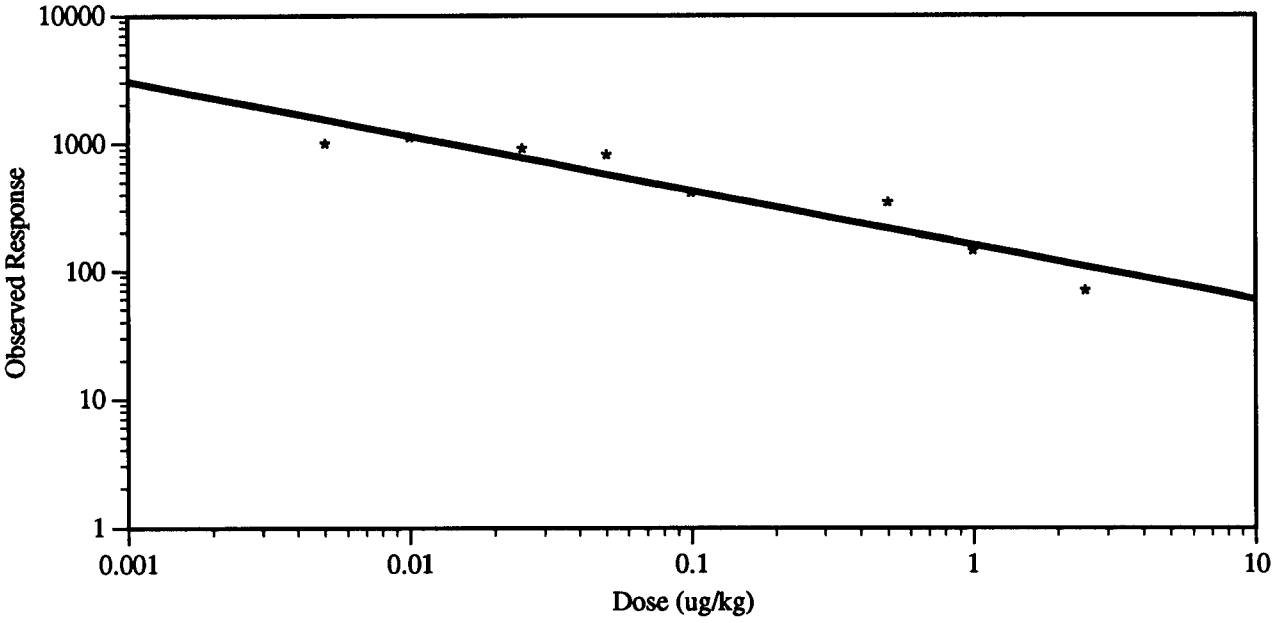
for

Female B6C3F1 mice , Narasimhan et al., 1993)

Response: number of PFC's/spleen

or % response PFCs/10^6 spleen cells

A

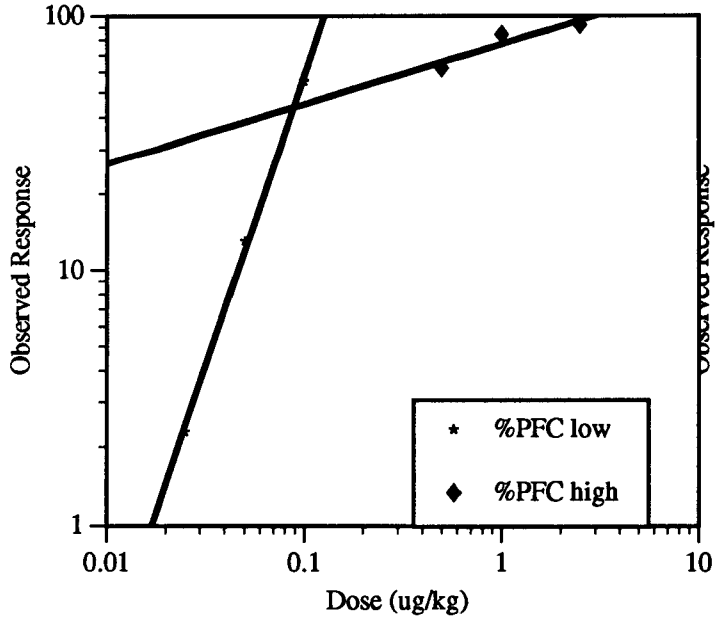


Functional Form of Dose-Response

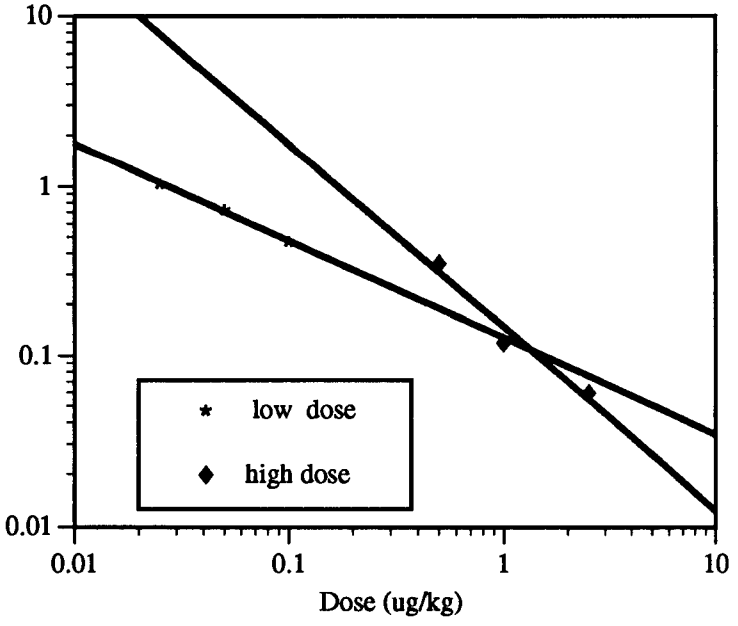
(Best Fit Estimate for Power Law)

f(x) =1.602198E+2 * x^-4.269049E-1, R^2 = 8.929944E-1

Dose -Response - Subsets - % response-B



Dose -Response - Subsets - raw data response-C



Functional Forms of Dose-% Response Curves

PFC low f(x) =1.164128E+4 * x^ 2.298986E+0, R^2 = 9.972033E-1

PFC high f(x) = 7.748043E+1*x^2.341332E-1, R^2 = 8.628811E-1

Functional Forms of Dose- Response Curves

PFC low f(x) = .274749E-1*(x^-5.729254E-1), R^2=9.961E-1

PFC high f(x)=1.474431E-1* (X^-1.078037E+0), R^2=9.594E-1

GRAPH C5

Comparative Dose-Response

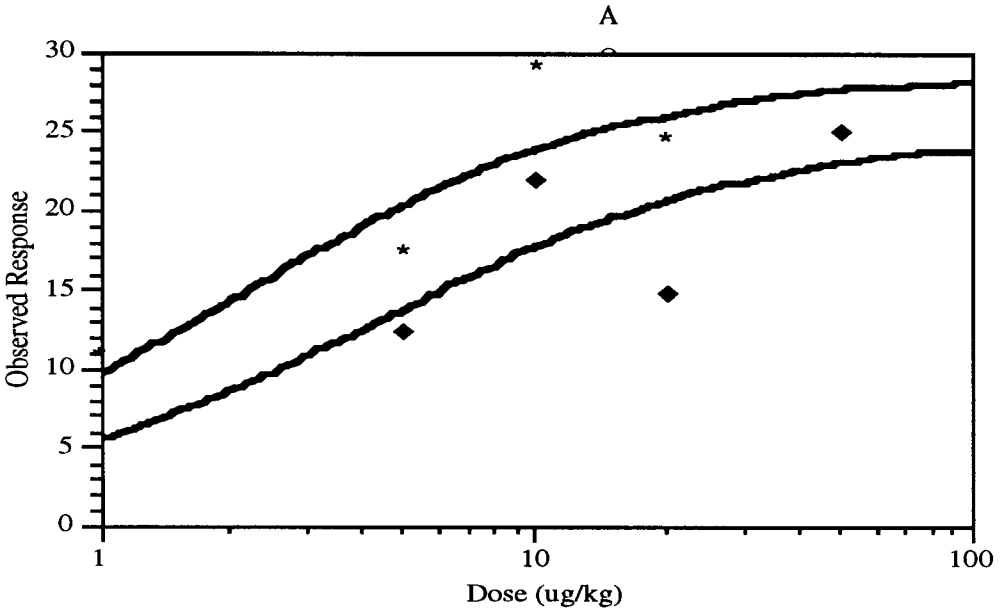
(Graph in Log-Log Coordinates)

C57BL/6J mice AhBB and Ahdd

(Kerkvleit et al. , 1990a)

Dose: single oral dose TCDD 2 days prior to an ip injection of 25 ug TNP-LPS

Response: decrease spleen cellularity

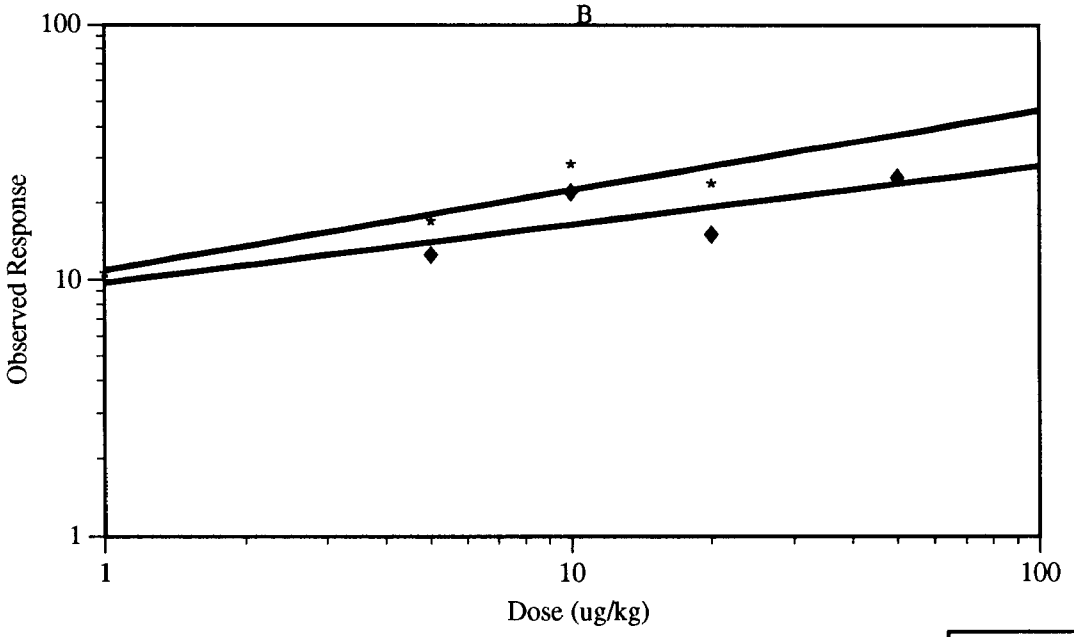


Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

Ahbb, f(x) =1+2.7767E+1*x/(2.1853353E+0+x), chi^2=38.15

Ahdd, f(x) =1+2.385456E+1*x/(4.32568E+0+x), chi^2=56.63



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

Ahbb: f(x) = 1.089295E+1*(3.140763E-1), R^2 = 8.527404E-1

Ahdd: f(x) = 9.726441E+1*(2.284170E-1), R^2 = 4.834202E-1

Graph D1

Comparative Dose-Response

(Graph in Log-Log Coordinates)f

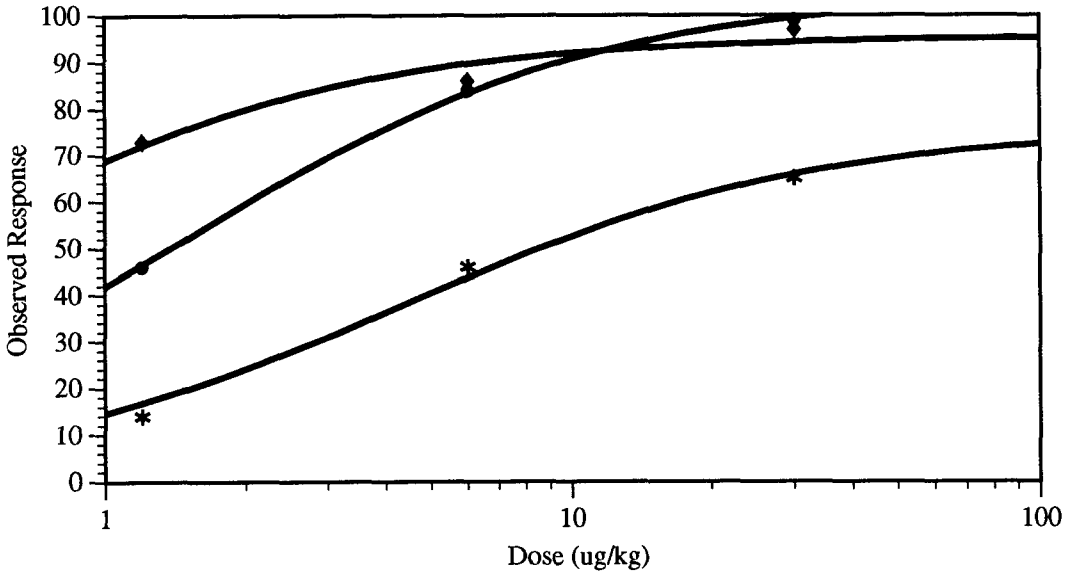
Male Mice differing in susceptiblility to Ah induction

(Vecchi, 1983)

Dose: single ip

Response: % decrease PFC/splenocytes at 5 days

A



Functional Forms of Dose-Response Curves

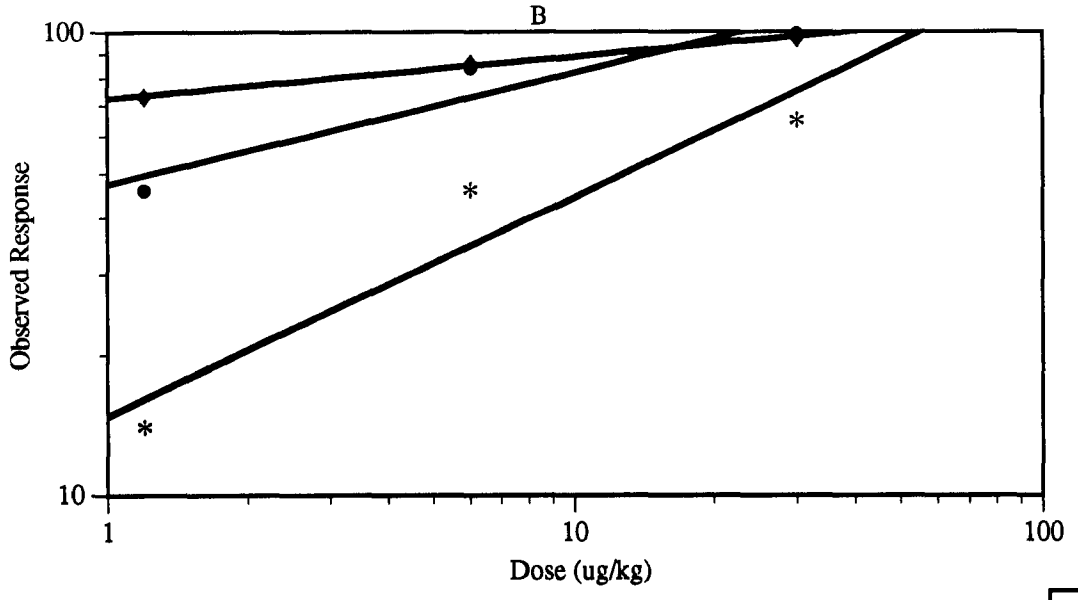
(Best fit Estimates for 1+ Michaelis-Menten)

C57BL/6:f(x) =1+9.448837E+1*x/3.957415E-1+x), chi^2=21.7

DBA: f(x) = 1+7.446390E+1*x/(4.459745E+0+x). chi^2=13.7

B6D2F1: f(x) = 1+1.034210E+2*x/(1.535931E+0+x), chi^2=0.71

B



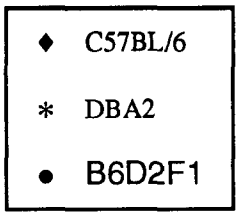
Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

C57BL/6: f(x) = 7.235706E+1 * (x^8.8.0771E-2), r^2 = 9.922455E-1

DBA2: f(x) = 1.477200E+1* (x^4.769771E-1), r^2 = 9.085196E-1

B6D2F1: f(x0 = 4.738026E+1 * (x^2.381199E-1),r^2 =9.018871E-1



GRAPH E

Dose-Response

(Graph in Log-Linear Coordinates)

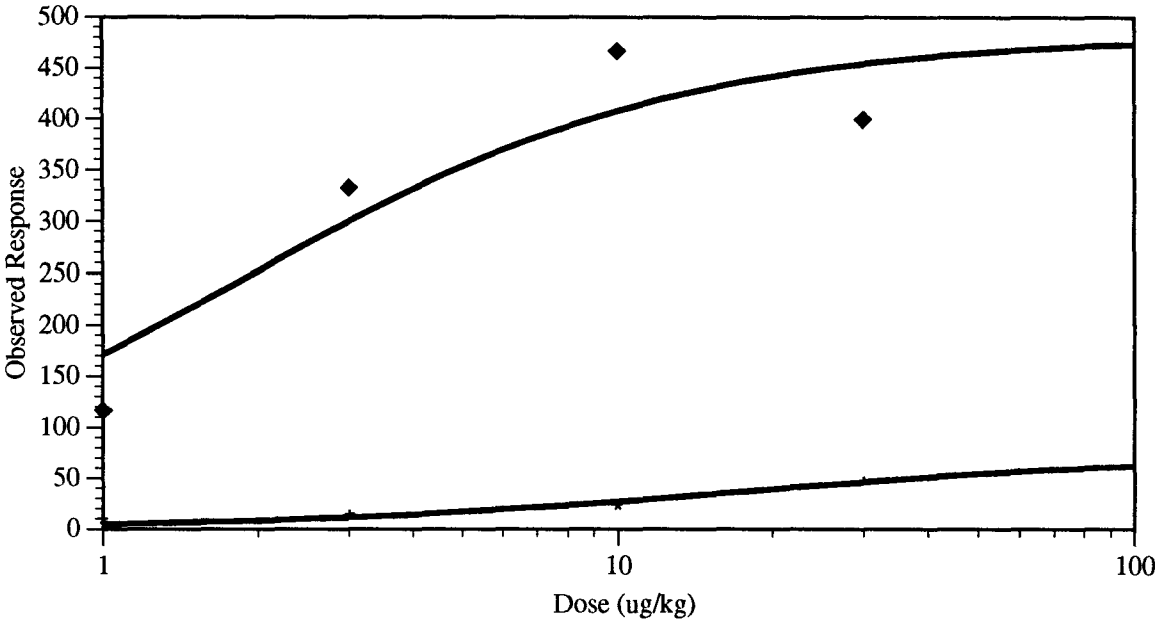
Female CD-1 mice

(DeVito et al. 1992))

Dose: single i.p.

Response: % decrease hepatic estrogen receptor increase hepatic AHH activity

A

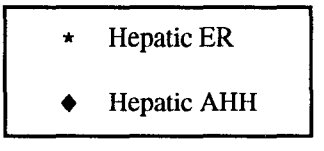


Functional Form of Sigmoid Dose-Response

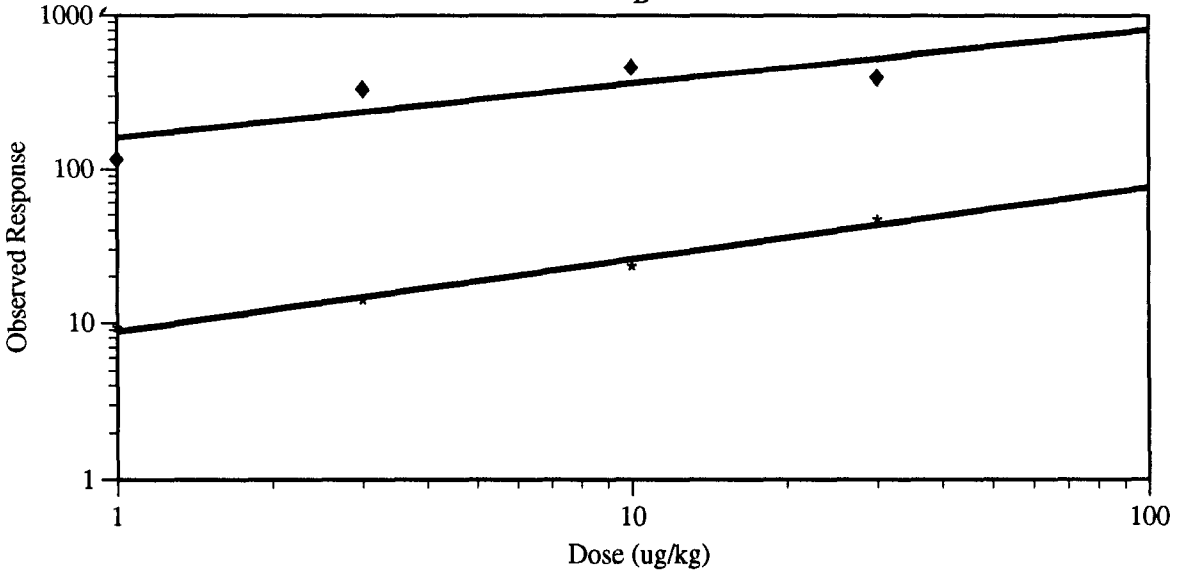
(Best Fit Estimate for "Michaelis-Menten" type response)

Hepatic AHH activity : f(x)=4.808068E+2*x/(1.821770E+0+x)

Hepatic ER: f(x) = 1.7.132724E+1*x/(1.761074E+1+x)



B



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

Hepatic ER: f(x) = 8.829252E+0*(x^4.667998E-1),R^2 = 9.842274E-1

Hepatic: f(x) = 1.600130E+2*(x^3.533556E-1),R^2 = 6.889952E-1(

GRAPH F

Dose-Response

(Graph in Log-Linear Coordinates)

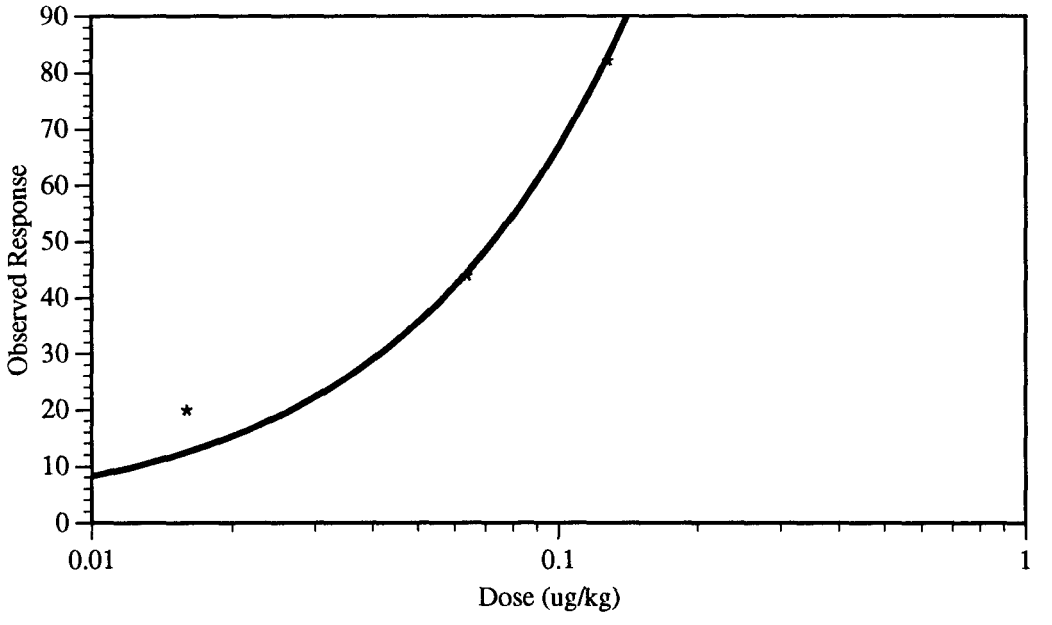
Female Sprague-Dawley Rats

(Astroff et. al, 1991)

Dose: single oral

Response: % decrease uterine c-fos mRNA units

A

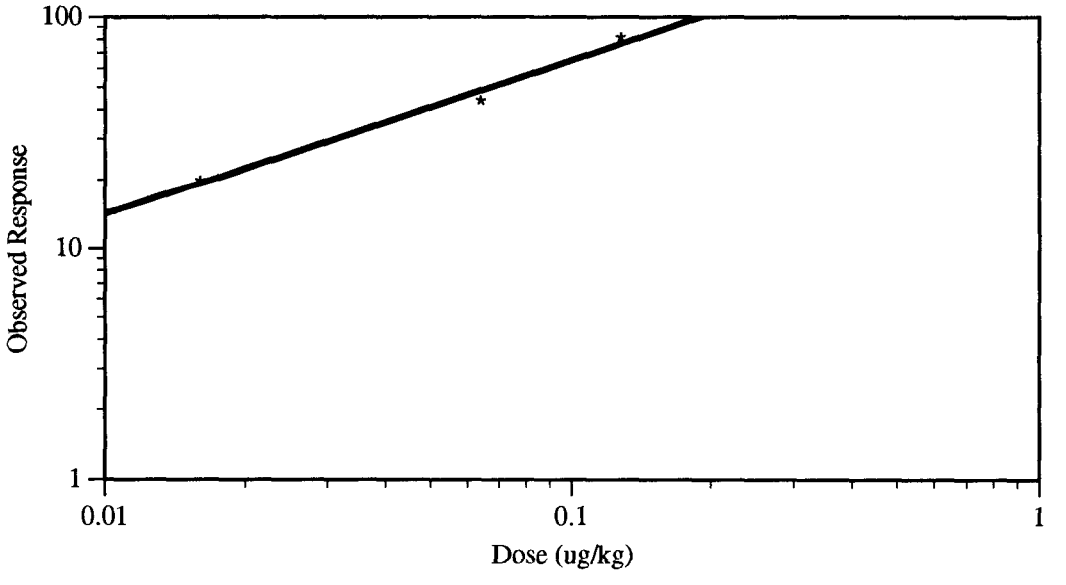


Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1 + Michaelis-Menten" type response)

f(x) =1+ 6.515702E+2*x/(8.920061E-1+x)

B



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

f(x) = 3.001080E+2* (x^6.628571E-1), R^2 = 9.851087E-1

Graph G

Dose-Response

(Graph in Log-Linear Coordinates)

for

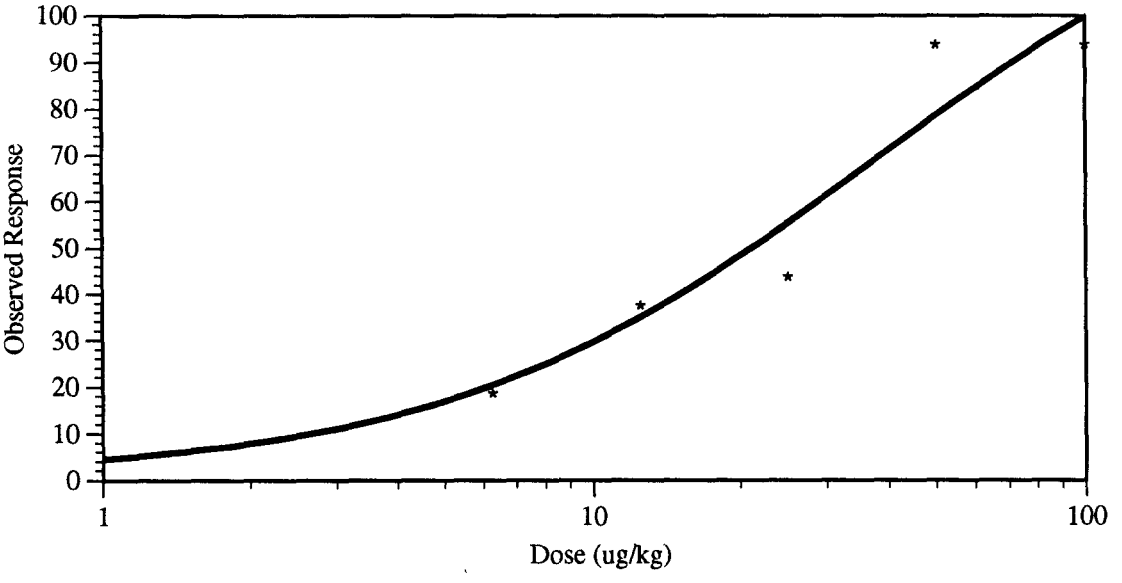
Male Sprague-Dawley Rats

(Moore et al, 1985)

Dose: single oral

Response: % decrease serum testosterone

A

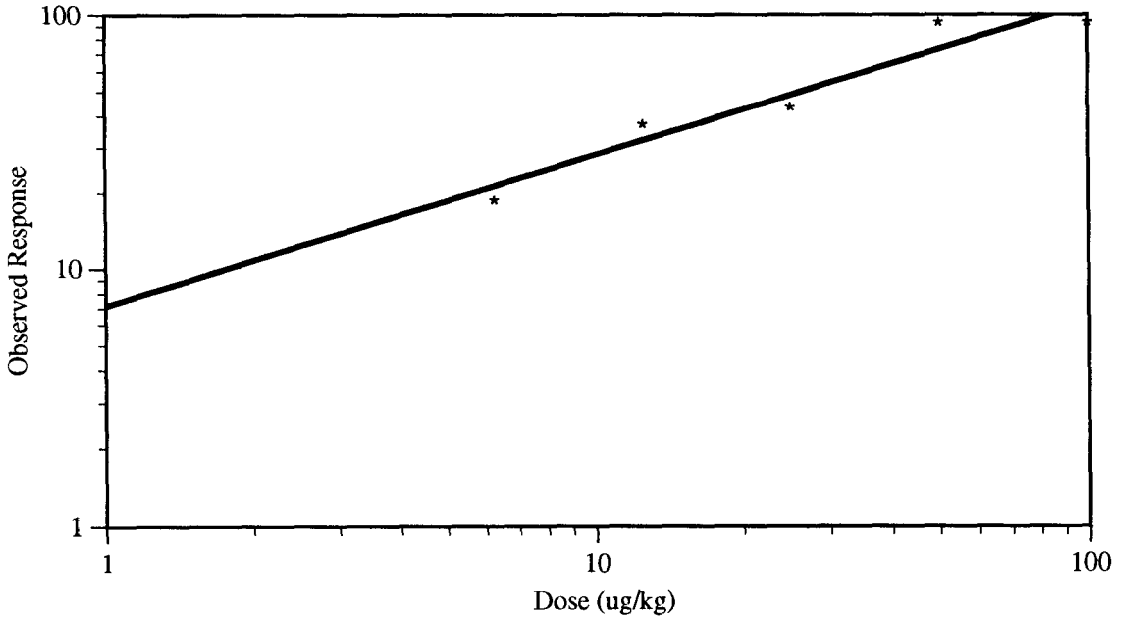


Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for Michaelis-Menten" type response)

f(x) = y=1+1.359828E+2*x/(3.753185E+1+x)

B



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

f(x) = 7.138131E+0 *(x^5.988408E-1),R^2 = 9.252290E-1

GRAPH H

Dose-Response

(Graphin Log-Linear Coordinates)

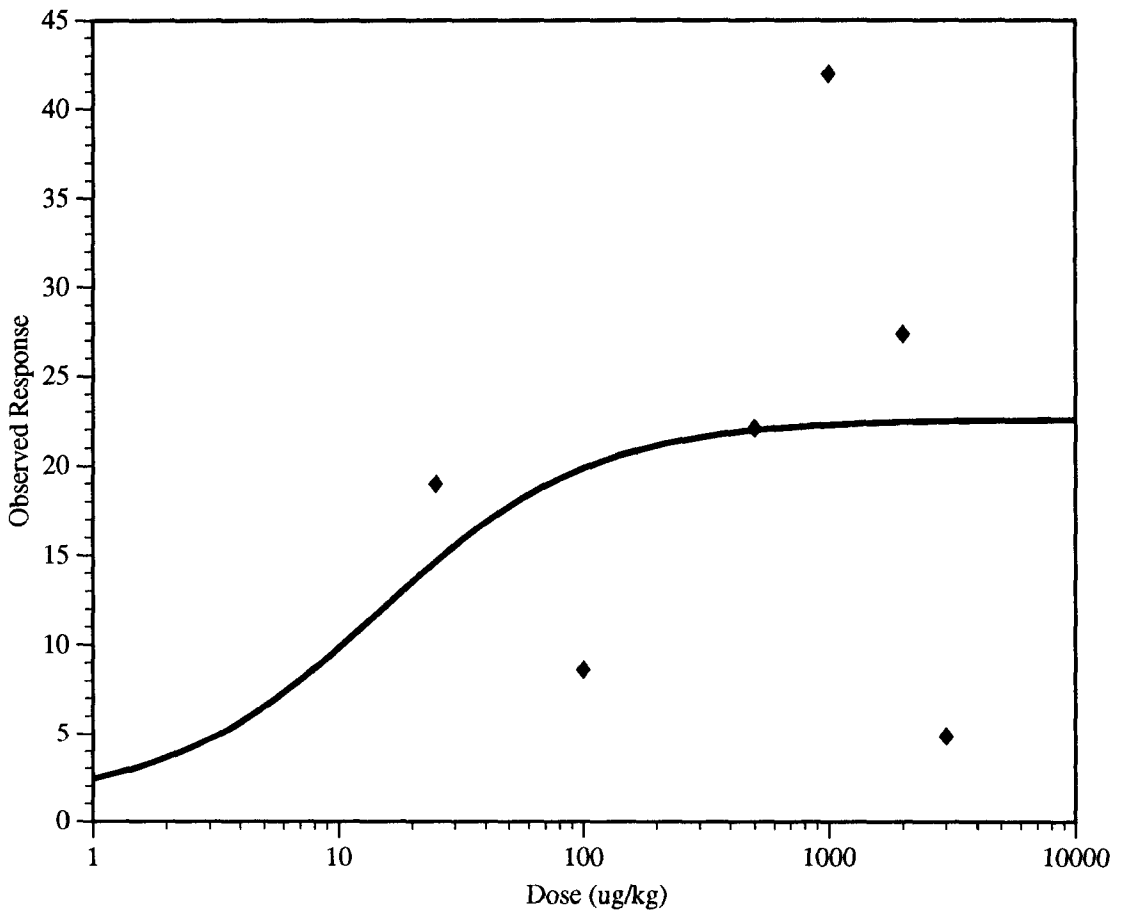
for

Female Syrian Hamsters

(Olsen et al, 1980)

Dose: single i.p.

Response: increase liver weight 50 days post dosing



Functional Form of Sigmoid Dose-Response

Best Fit Estimate for "1 + Michaelis-Menten" type response)

f(x) = 1 +2.156705E+1 *x/(1.447850E+1+x)

GRAPH I

Comparative Dose-Response

(Graph in Log-Log Coordinates)

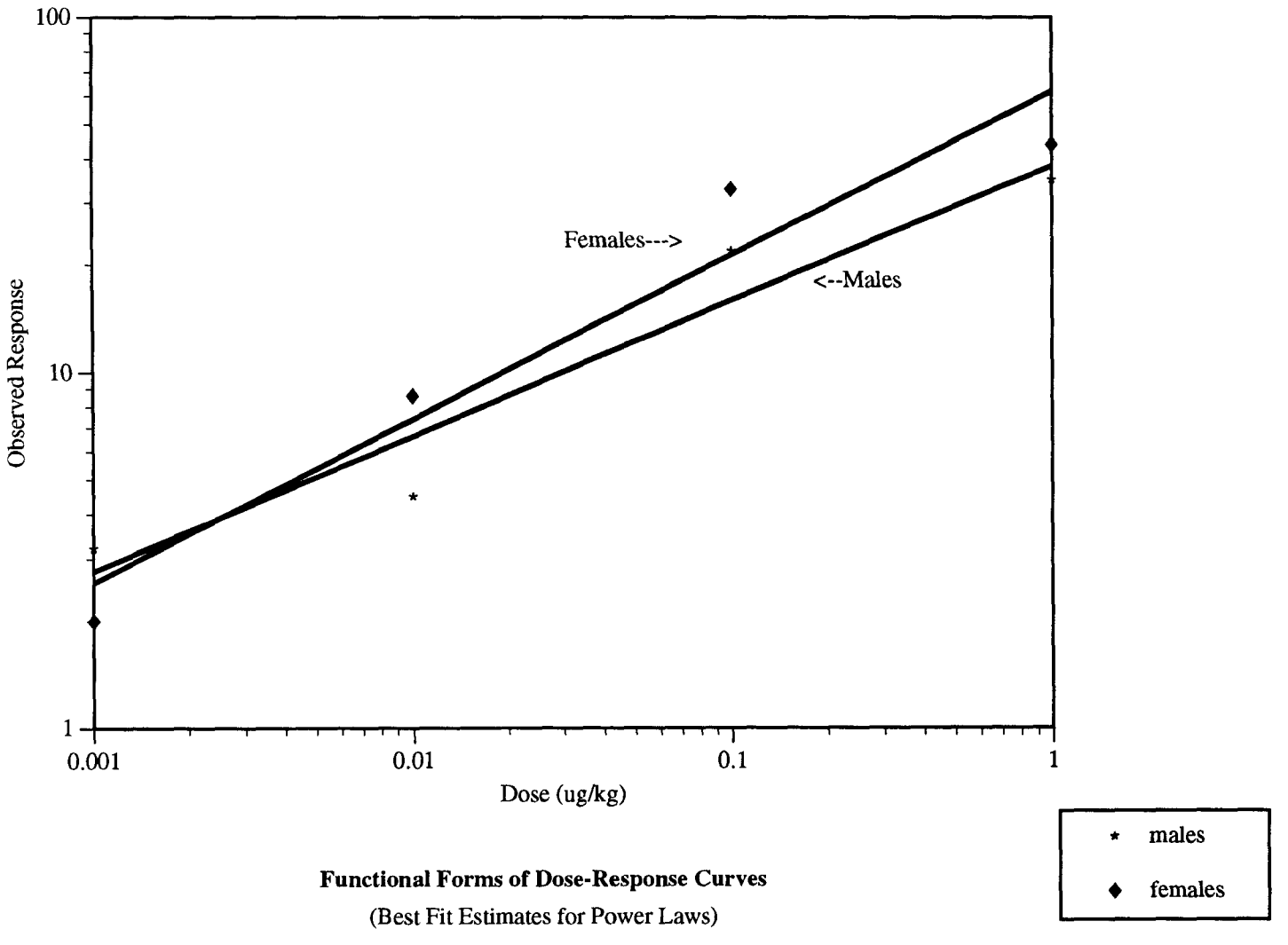
for

Female and Male Sprague-Dawley Rats

(Kociba, 1976)

Dose: p.o. 5x/wk 13 weeks

Response: increase liver/bwt ratio



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

Female: f(x) = 6.181107E+1 * (x^4.611284E-1),R^2 = 9.357175E-1

Male: f(x) = 3.820392E+1 * (x^3.805964E-1), R^2 = 9.310575E-1

Graph J1

Dose-Response

(Graph in Log-Linear Coordinates)

for

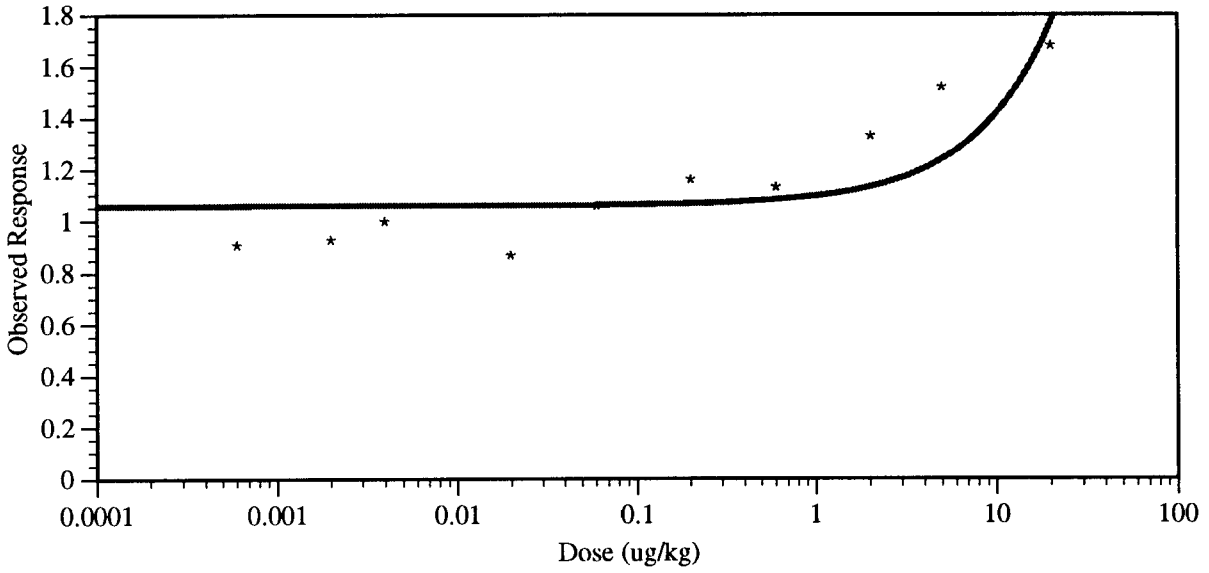
Female Sprague-Dawley Rats

(Kitchin and Woods, 1979)

Dose: single oral

Response: raw data change in P-450 at 3 days

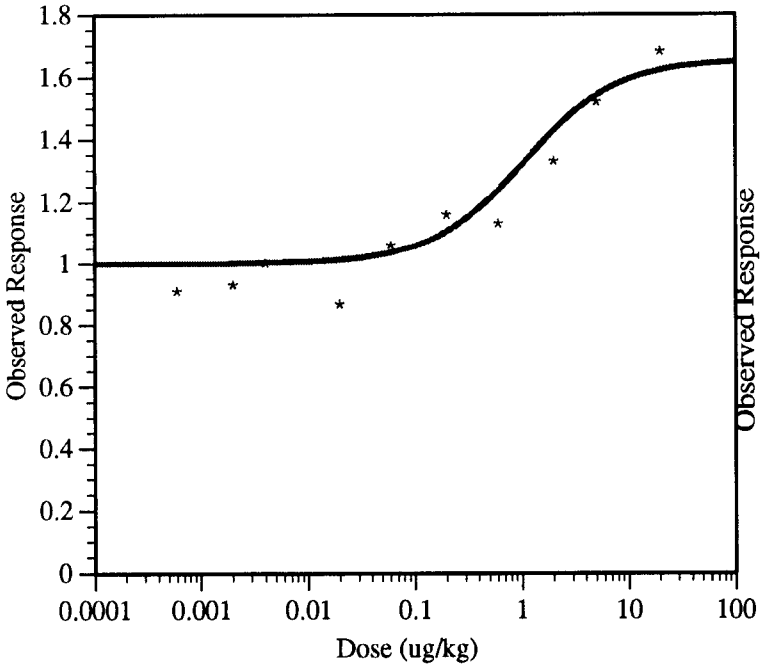
A



Functional form Linear Dose-Response Best Fit

f(x) =3.565270E-2*x+1.05977E+0, R^2=6.72222497E-1

B

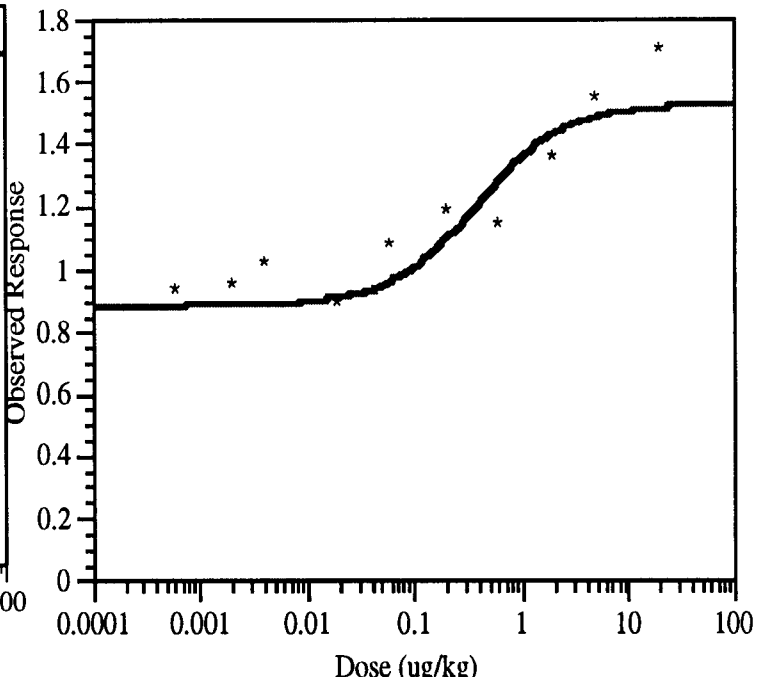


"Functional Form of Sigmoid Dose-Response

Best fit for Michaelis-Menten + background paramc ter response)

f(x) = y=0.88 + 7.812964E-1*x/(1.022375E+0+x)

C



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill function plot

f(x)=(6.466629E-1*x^1.08926E+0)/(3.584389E1+

x^1.089267E+0)+0.88; chi square = 9.346E-2

GRAPH J2

Comparative Dose-Response

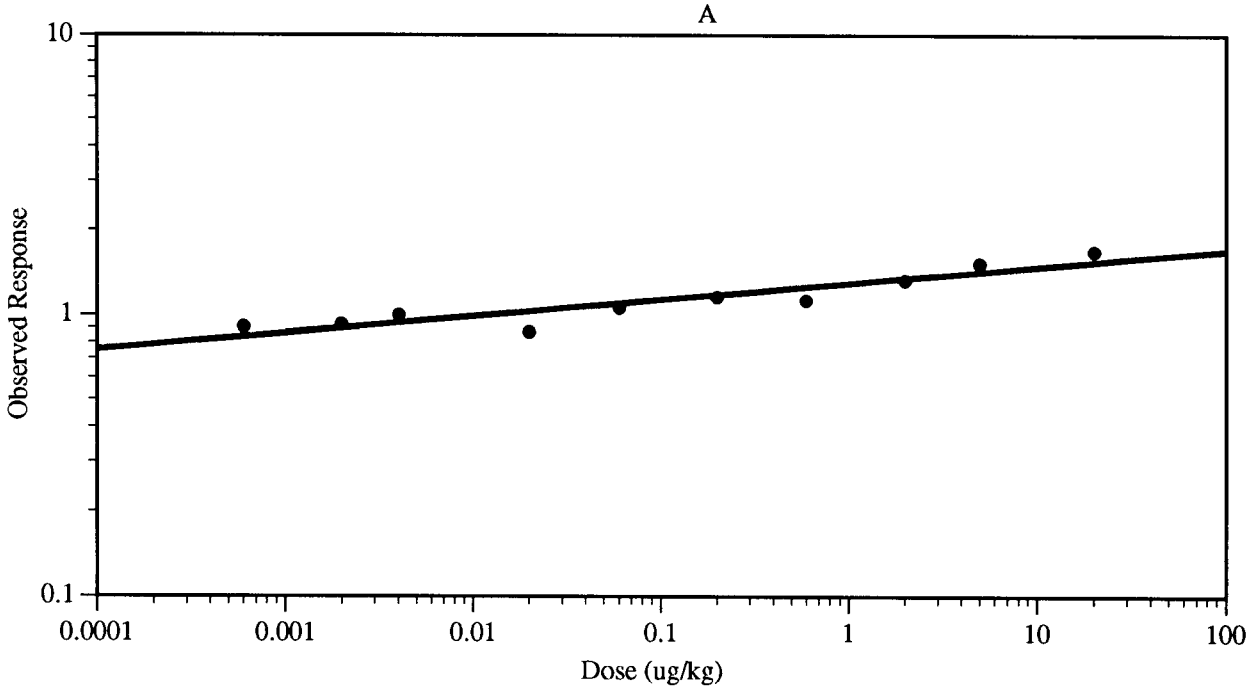
(Graph in Log-Linear Coordinates) for

Female Sprague-Dawley Rats (Kitchin and Woods, 1979)

Dose: single oral

Response: raw data P450 (3 days)

A

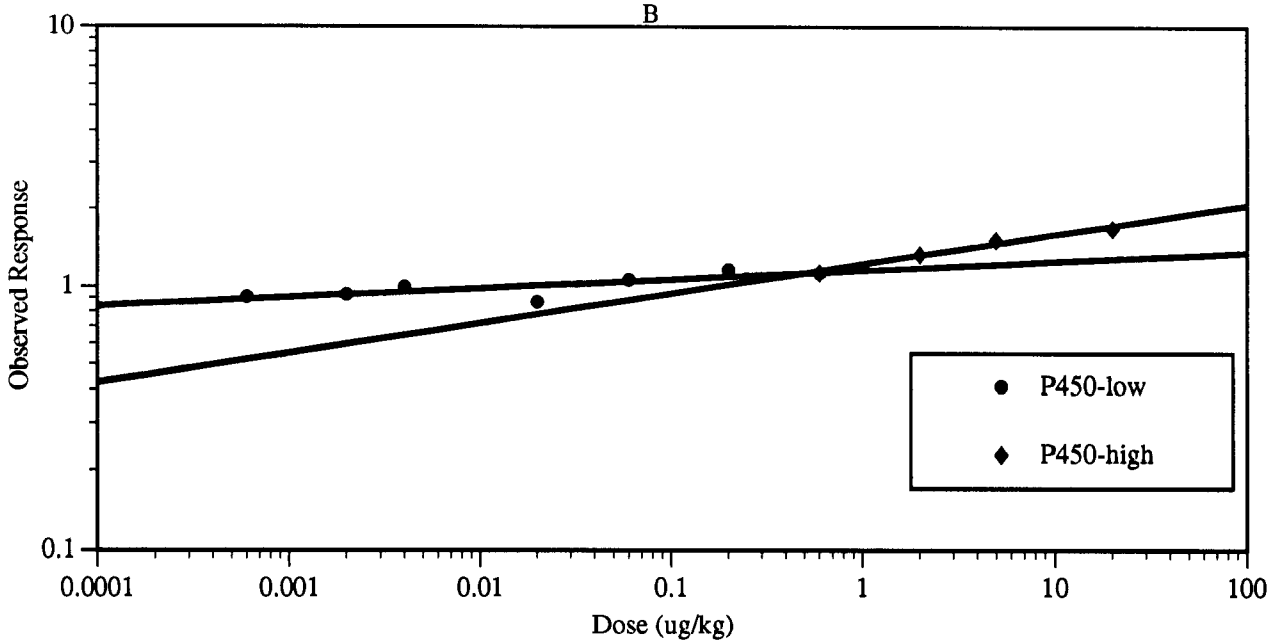


Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

complete data set f(x) = 1.293664E+0 * (x^5.855594E-2), R^2 = 8.538984E-1

B



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

(.0006 to .6 ug/kg) f(x) = 1.152077E+0 *(x^3.457812E-1), R^2 = 6.187106E-1

(0.6 to 20 ug/kg) f(x) = 1.220259E+0 * (x^1.146001E-1), R^2=9.754329E-1

Graph K1

Dose-Response

(Graph in Log-Linear Coordinates)

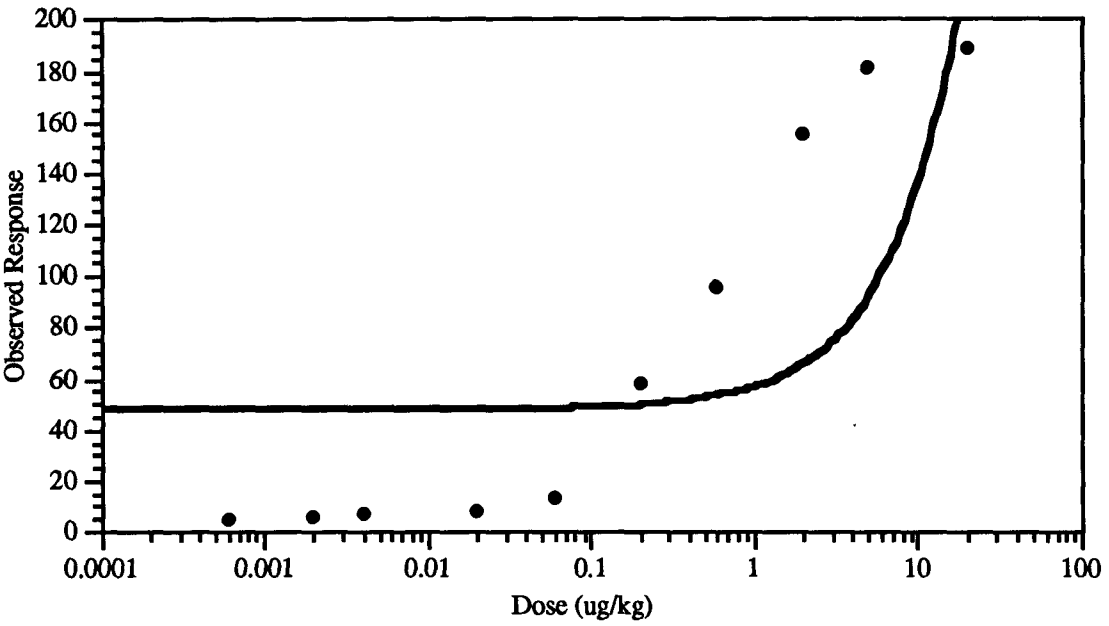
Female Sprague-Dawley Rats

(Kitchin, 1979)

Dose: single oral

Response: increase Benzo(a) pyrene hydroxylase raw data

A



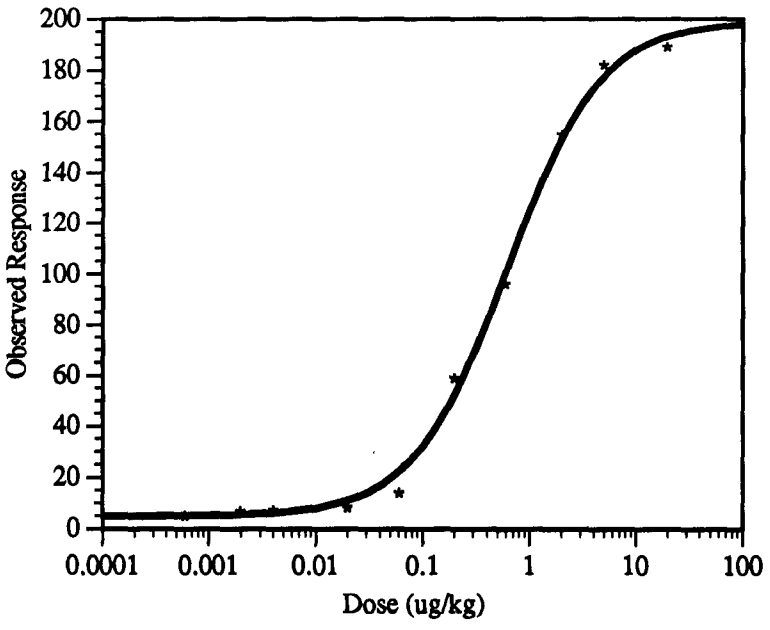
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Linear Model" type response)

f(x) = 8.692922E+0*x +4.796840E+1

R^2 = 4.938167E-1

B



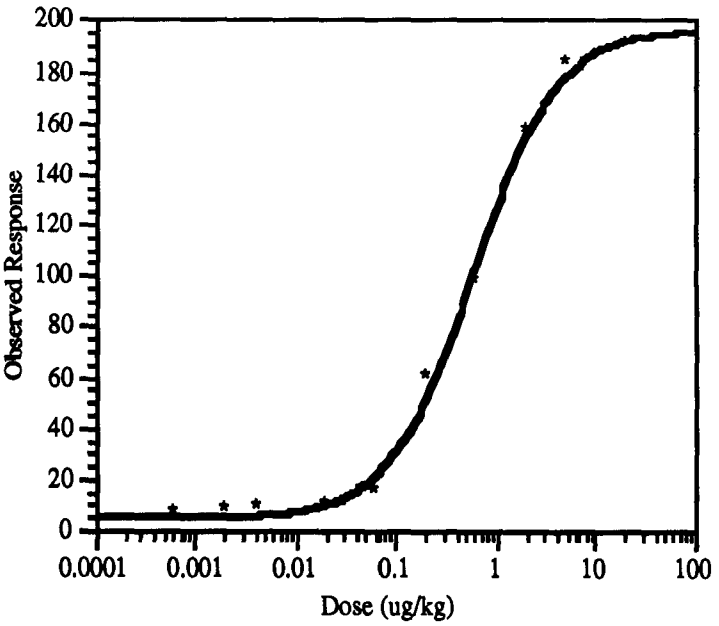
Functional Form of Sigmoid Dose-Response

(Best Estimate for "Michaelis-Menten + background parameter")

f(x) = y=4.9 +1.939516E+2*x/(6.164672E-1+x),

chi^2 = 197.2

C



Functional Form of Sigmoid Dose-Response

(Best for " Hill Function" + background parameter)

f(x) =1.0909172E+2 *x^1.066342E+0/ 5.697987E-1 + x^1.066342E+0)

chi^2 = 163

K2

Comparative Dose-Response

(Graph in Log-Log Coordinates)

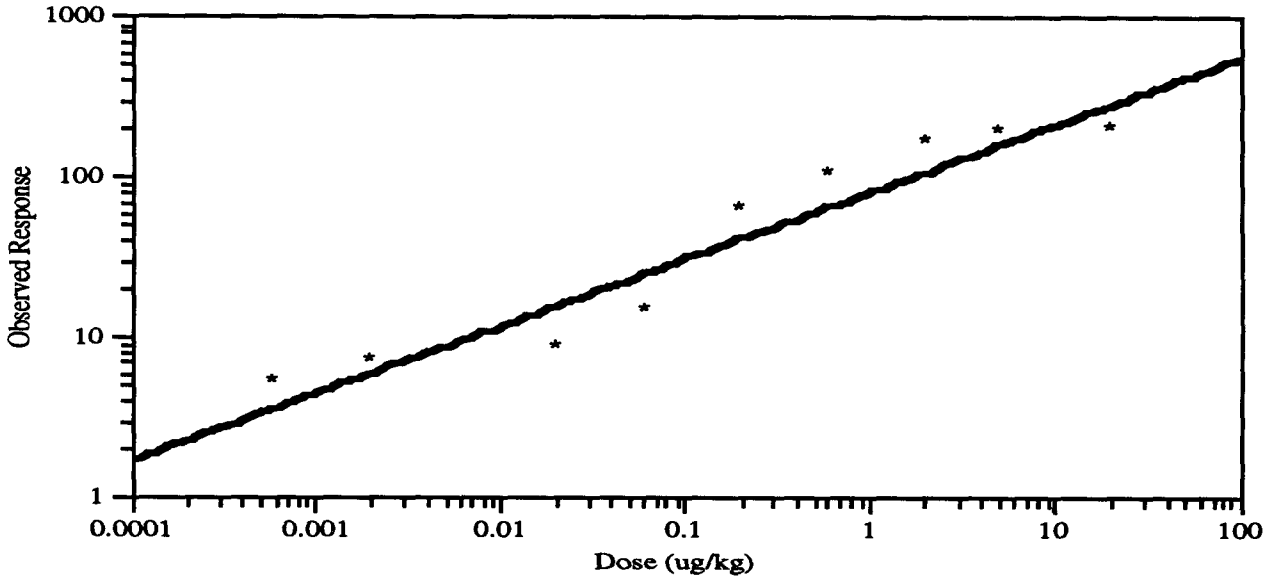
Female Sprague-Dawley Rats

(Kitchin and Woods. 1979)

Dose: single oral

Response: increase Benzo(a) pyrene hydroxylase

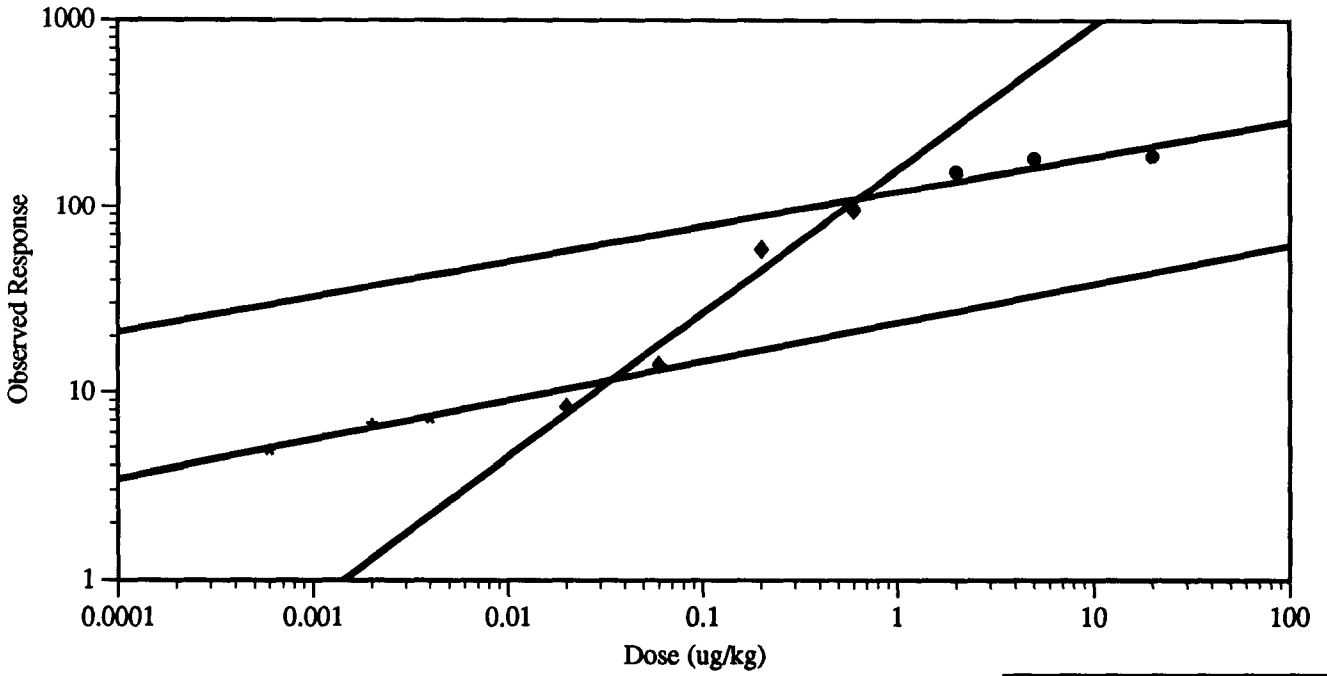
A



Functional Form of Dose-Response

(Best Fit Estimate for Power Law)

f(x) =8.044850E+1 * (x^4.195383E-1), R^2 = 9.310005E-1



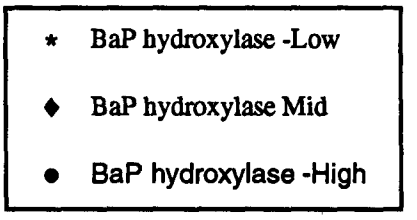
Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

(x = 0.0006 to 0.004) f(x) = 2.351223E+1*(x^2.091992E-1) ,R^2 = 9.633734E-1)

(x = 0.02 to 0.6)f(x} = 1.572746E+2 * (x^7.726666E-1) , R^2 = 9.638735E-1

(x = 0.6 to 20)f(x) = 1..2000001E+2 * x^1.887329E-1),R^2 = 8.048891E-1)



Graph L1

Dose-Response

(Graph in Log-Linear Coordinates)

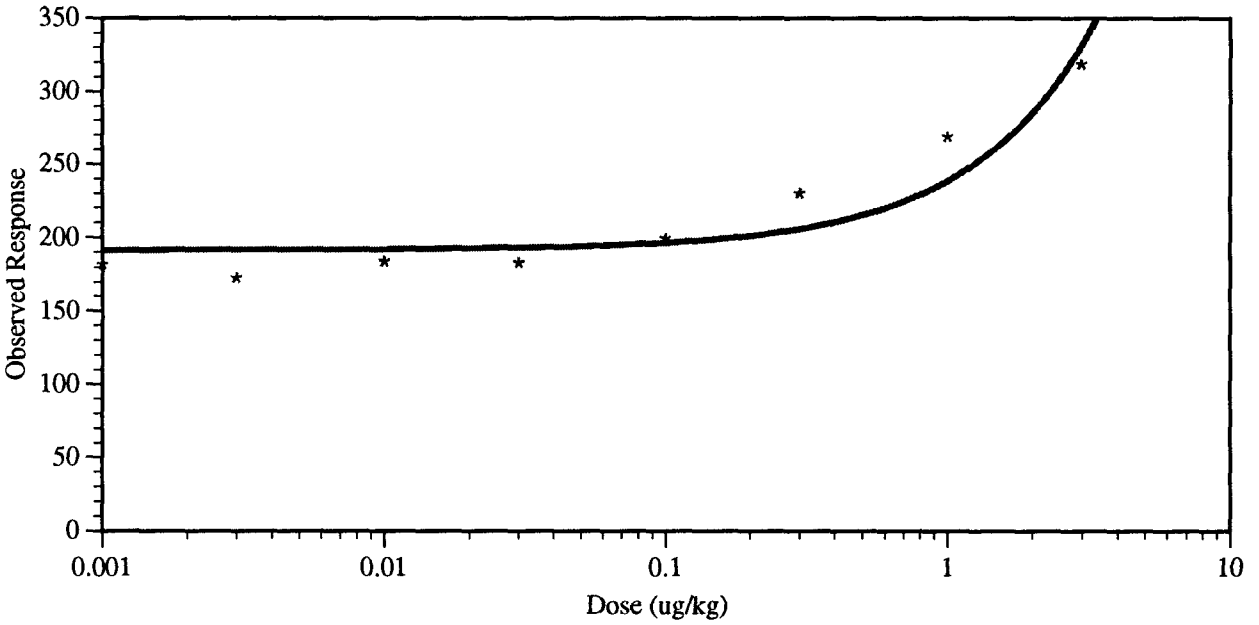
Female Wistar Rats

(Abraham, 1980)

Dose: single sc

Response raw data P450 at 7 days

A

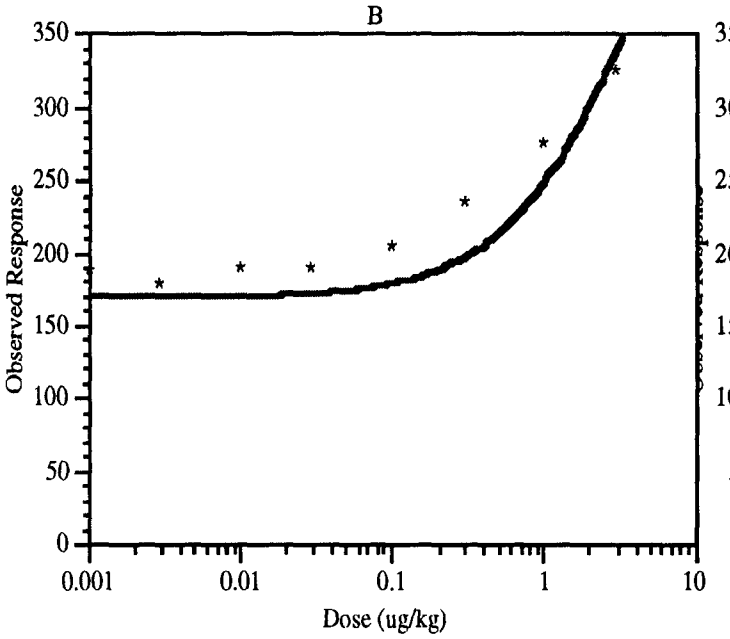


Functional Form of Dose-Response

(Best Fit Estimate for Linear Model)

f(x) =4.676353E=1 * x+1.913979E+2, R^2 = 8.781851E-1

B



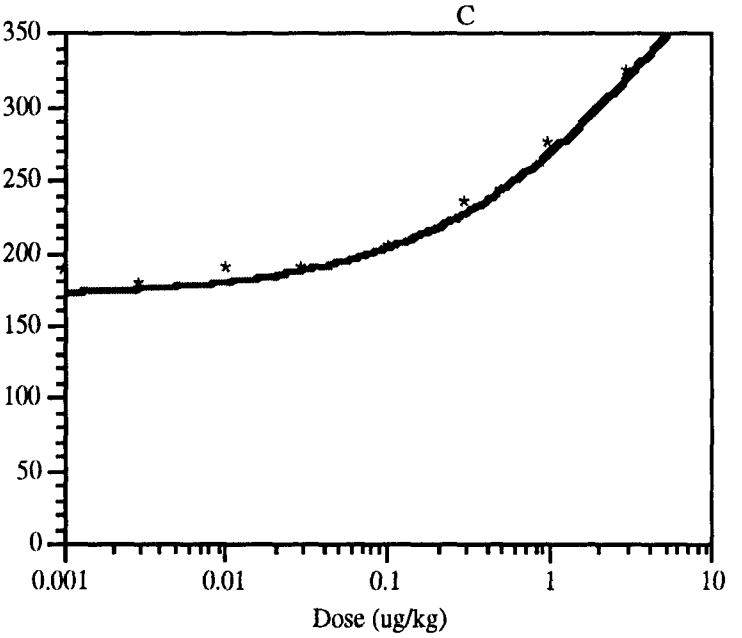
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for " Michaelis-Menten + background parameter"

f(x) = y= 169 + 3.876606E+2*x/(3.886855E+0+x)

Chi^2 =2769

C



Functional Form of Dose-Response

(Best Fit Estimate for Hill funcction + background parameter)

f(x) = 3.994308E+2*x^5.478666E-1/(3.021723E+0+

x^5.478666E-1) + 169, Chi^2 =169

Graph L2

Dose-Response

(Graph in Log-LogCoordinates)

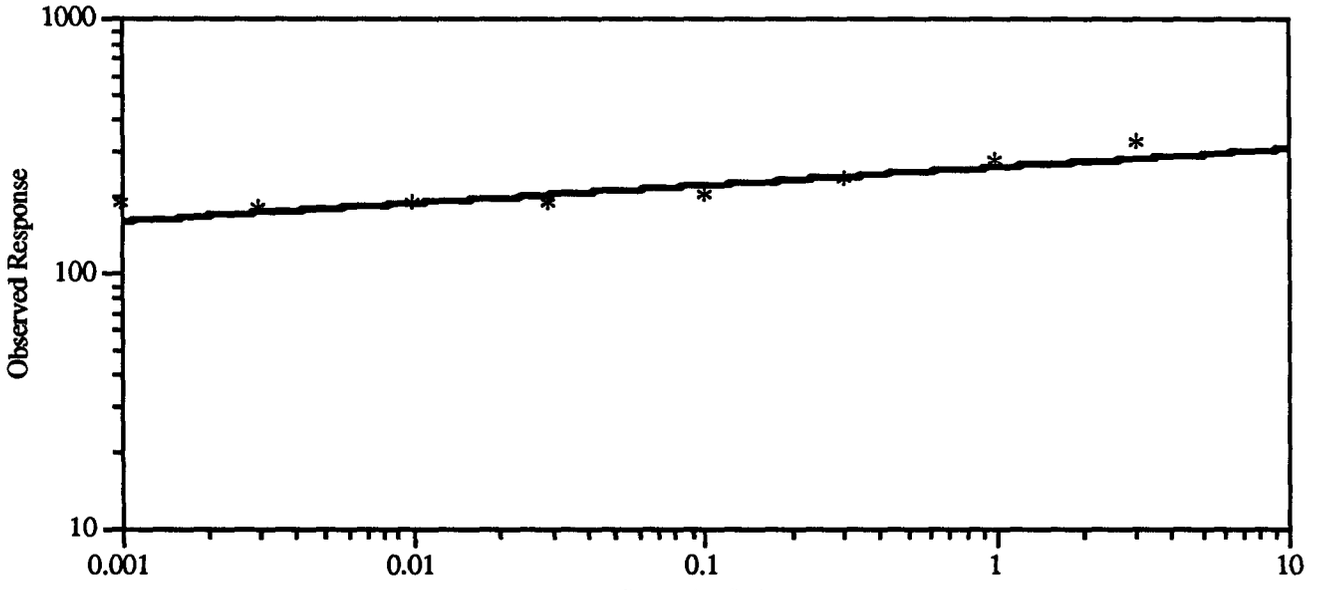
Female Wistar Rats

(Abraham et al., 1980)

Dose: single sc

Response: raw data: P450

A

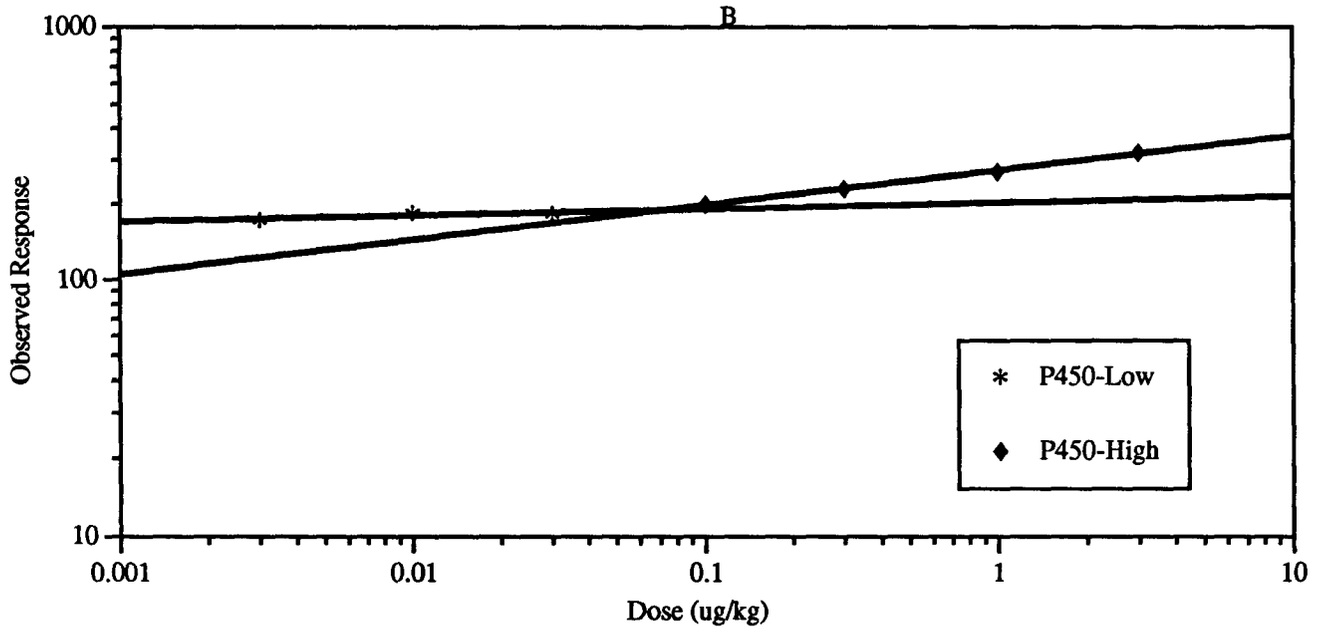


Functional Form of Dose-Response

(Best Fit Estimate for Power Law)

f(x) = 2.615298E+2* (x^7.141240E-2), R^2 = 8.300624E-1

B



Functional Form of Dose-Response

(Best Estimate for Power Law)

P450-Low f(x) = 2.134553E+2*(x^3.591655E-2), R^2 = 8.762258E-1

P450-High f(x) =2.719892E+2*x^1.377786E-1, R^2 = 9.982764E-1

Graph M1

Dose-Response

(Graph in Log-Linear Coordinates)

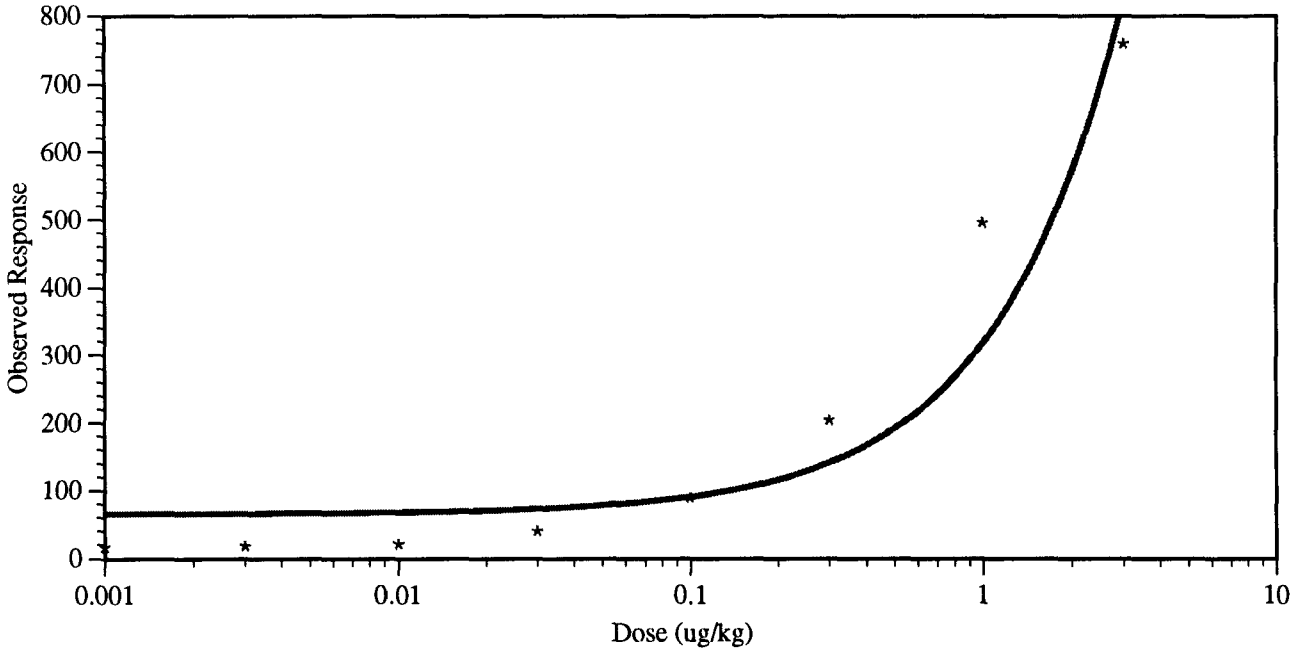
Female Wistar Rats

(Abraham et al., 1980)

Dose: single sc

Response: raw data: EROD - 7 days

A

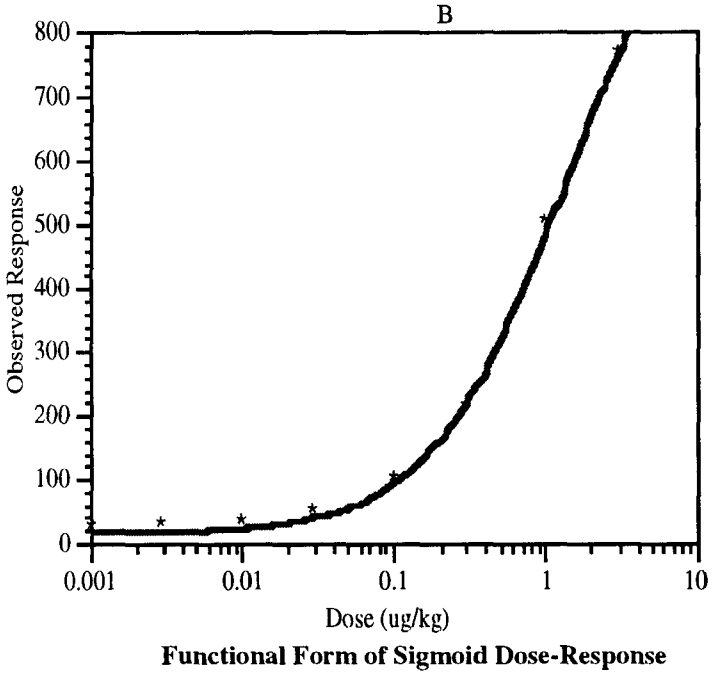


Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Linear "type response)

f(x) = 2.527667E+2*x + 6.538808E+1, R^2 = 0.911

B

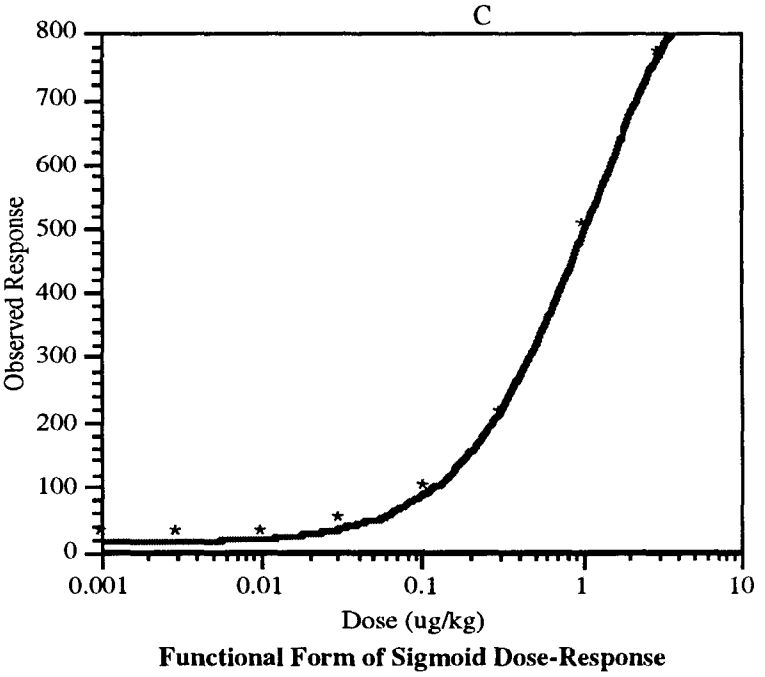


Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" + background parameter" type response)

f(x) =14.3 + 1.066009E+3*x/(1.273480E+0+x), Chi^2 = 343

C



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill plot with background parameter')

f(x)=(9.877489E+2*x^1.088899E+0)/1.072719E+0+ x^1.088899E+0)+ 14.3, Chi^2 = 176

Graph M2

Comparative Dose-Response

Dose-Response

(Graph in Log-Log Coordinates)

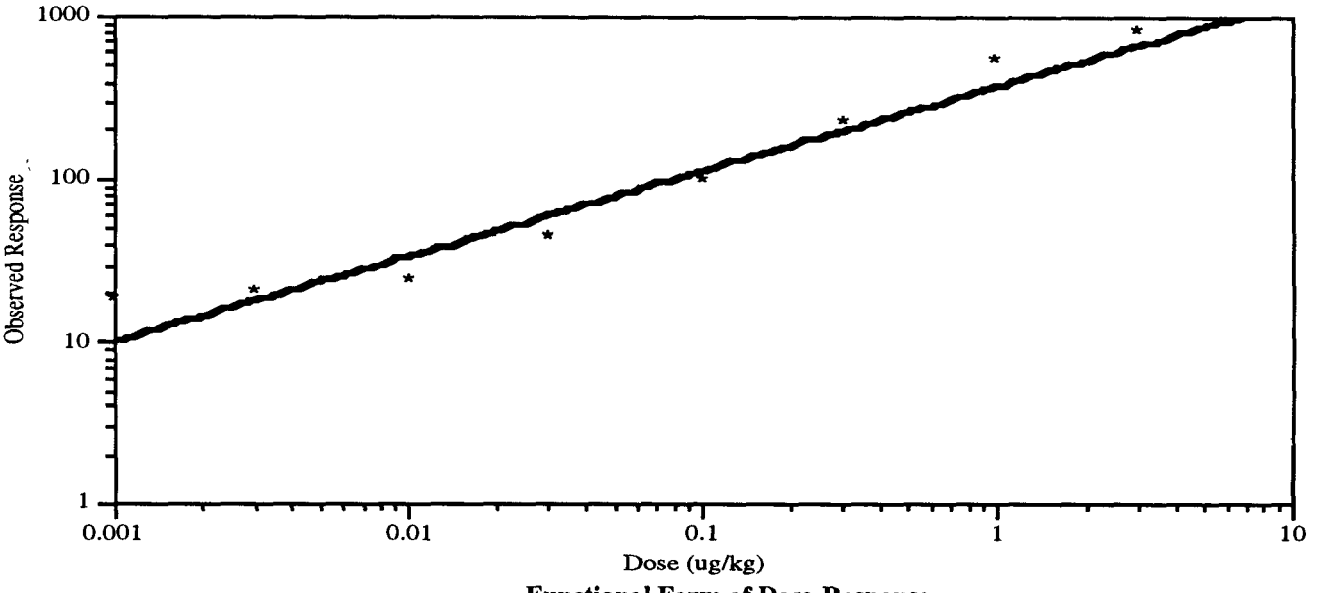
Female Sprague-Dawley Rats

Abraham et al., 1988

Dose: single sc

Response: raw data EROD (7 days)

A

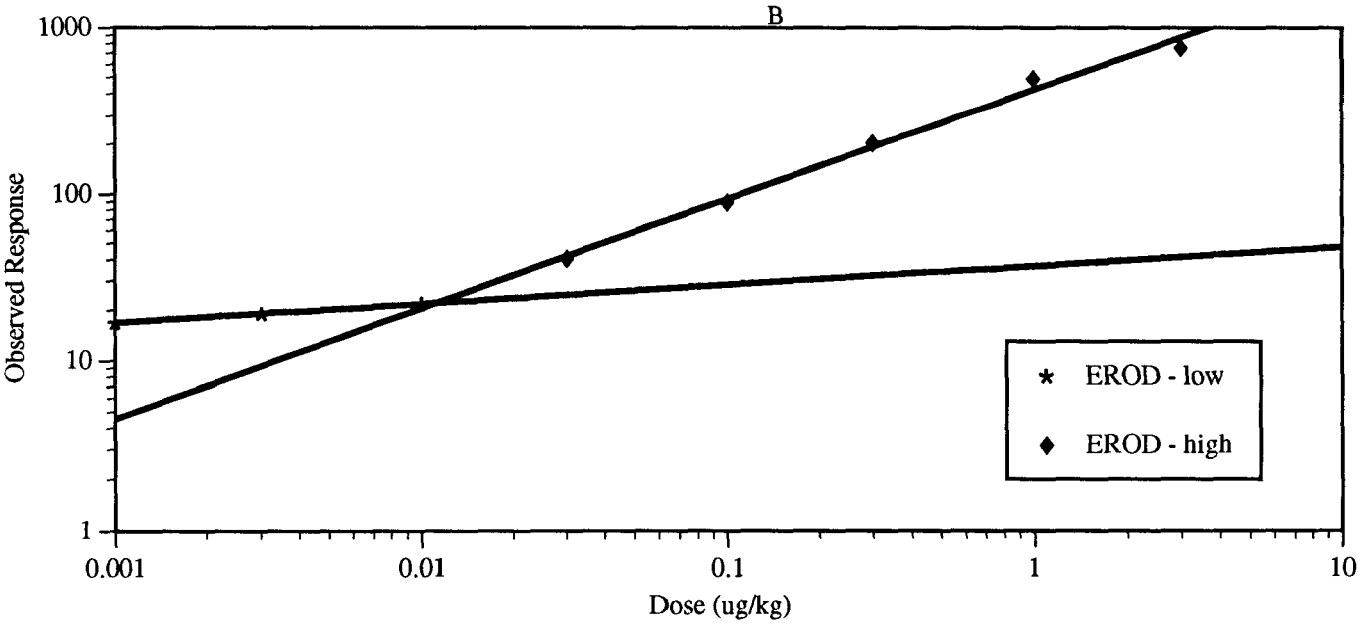


Functional Form of Dose-Response

(Best Fit Estimate for Power Law)

f(x) = 3.724271E+2 *(x^5.226669E-1) , R^2 =9.528637E-1

B



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

EROD - low f(x) = 3.671388E+1 *(x^1.126439E-1), R^2 = 9.991464E-1

EROD - high f(x) =4.236494E+2 * (x^6.572701E-1), R^2 = 9.912442E-1

GRAPH N.

Comparativ Dose-Response

(Graph in Log-Log Coordinates)

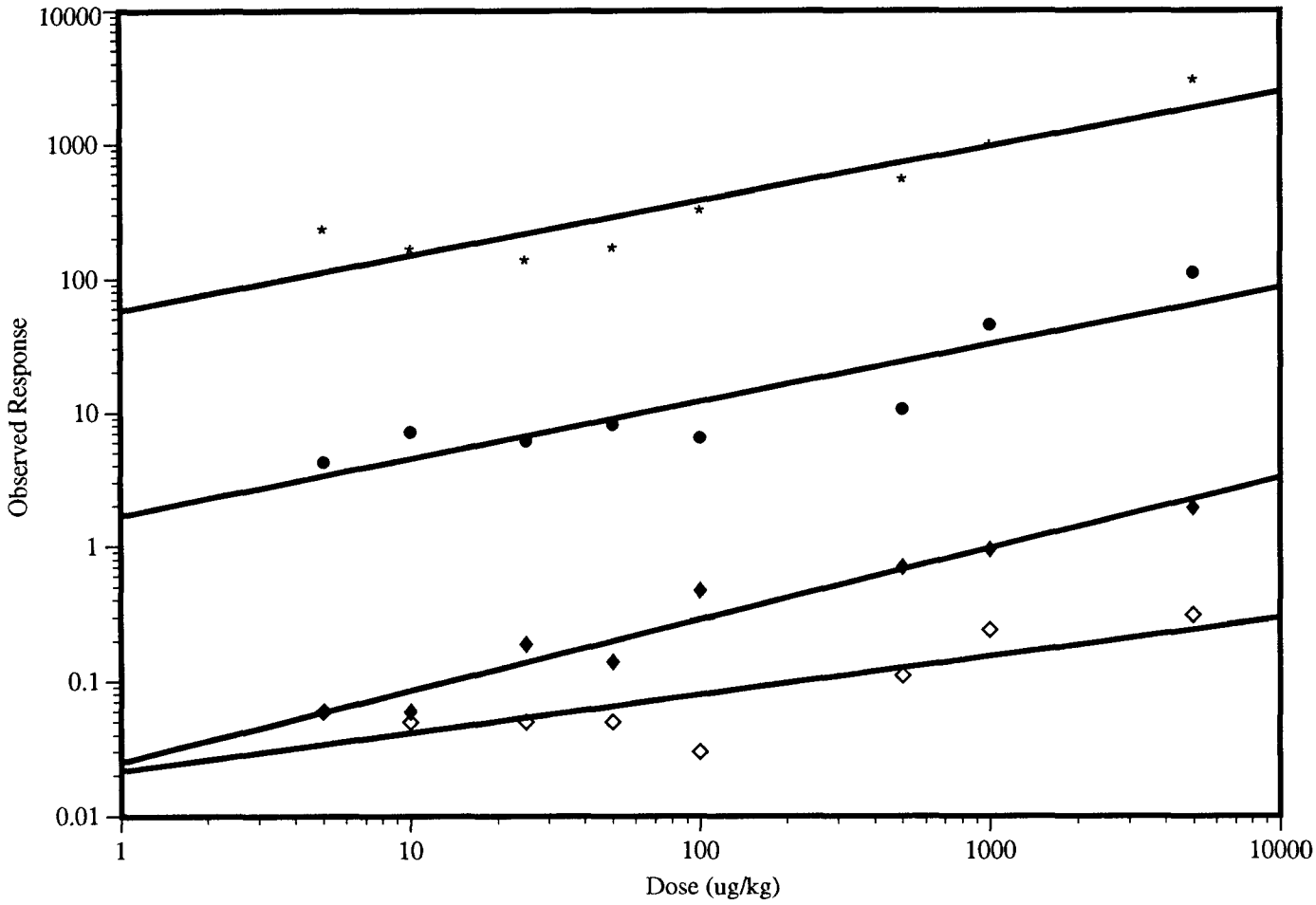
for

Female B6C3F1 Mice

(Safe 1993)

Dose: single i.p. injection

Response: 3 days post dosing increase EROD, Cyp1A1,Cyp1A2, Total nuclear Ah Receptor Binding



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

EROD f(x) = 5.810675E+1 * (x^4.071389E-1), R^2 = 8.276920E-1

Cyp1A1 f(x) 2.529055E-2 * (x^5.273765E-1), R^2 = 9.472254E-1

Total Binding f(x) = 1.694760E+0 * (x^4.264534E-1), R^2 = 8.042876E-1

Cyp1A2 f(x) =2.169244E-2*(x^2.824955E-1), R^2 = 6.557616E-1

* EROD pmol/mg/min

♦ Cyp1A1 mRNA

● total receptor binding

◇ Cyp1A2

GRAPH O

Comparative Dose-Response

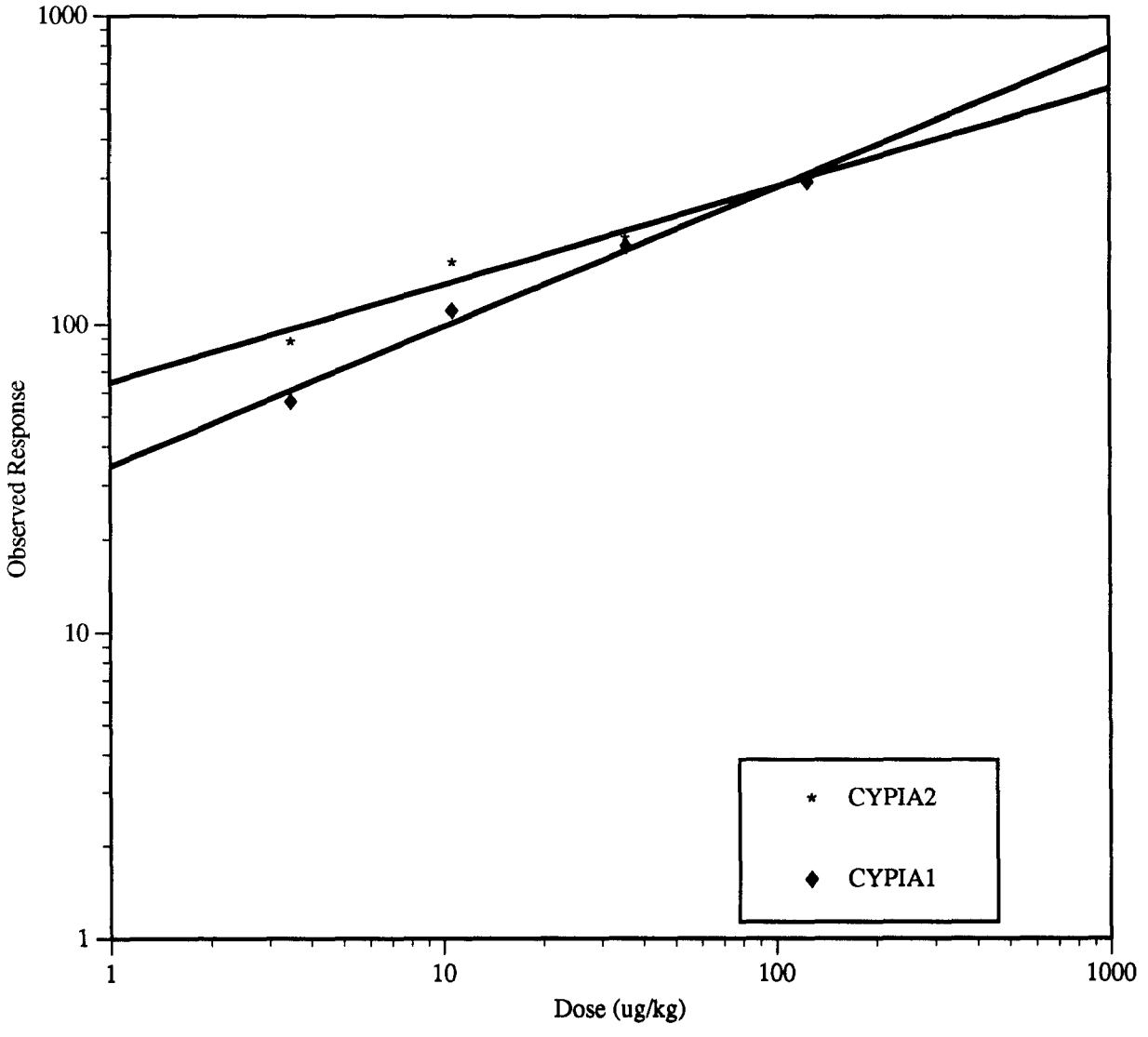
(Graph in Log-Log Coordinates)

Female Sprague-Dawley Rats

(Trischer et al., 1992)

Dose: p.o. biweekly for 30 weeks

Response: increase enzyme activity



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

CypIA1 f(x) = 6.47089E+1 * (x^3.193647E-1), R^2 = 9.546921E-1

CypIA2 f(x) = 3.453639E+1 * (x^4.540268E-1), R^2 = 9.869648E-1

GRAPH P1

Comparative Dose-Response

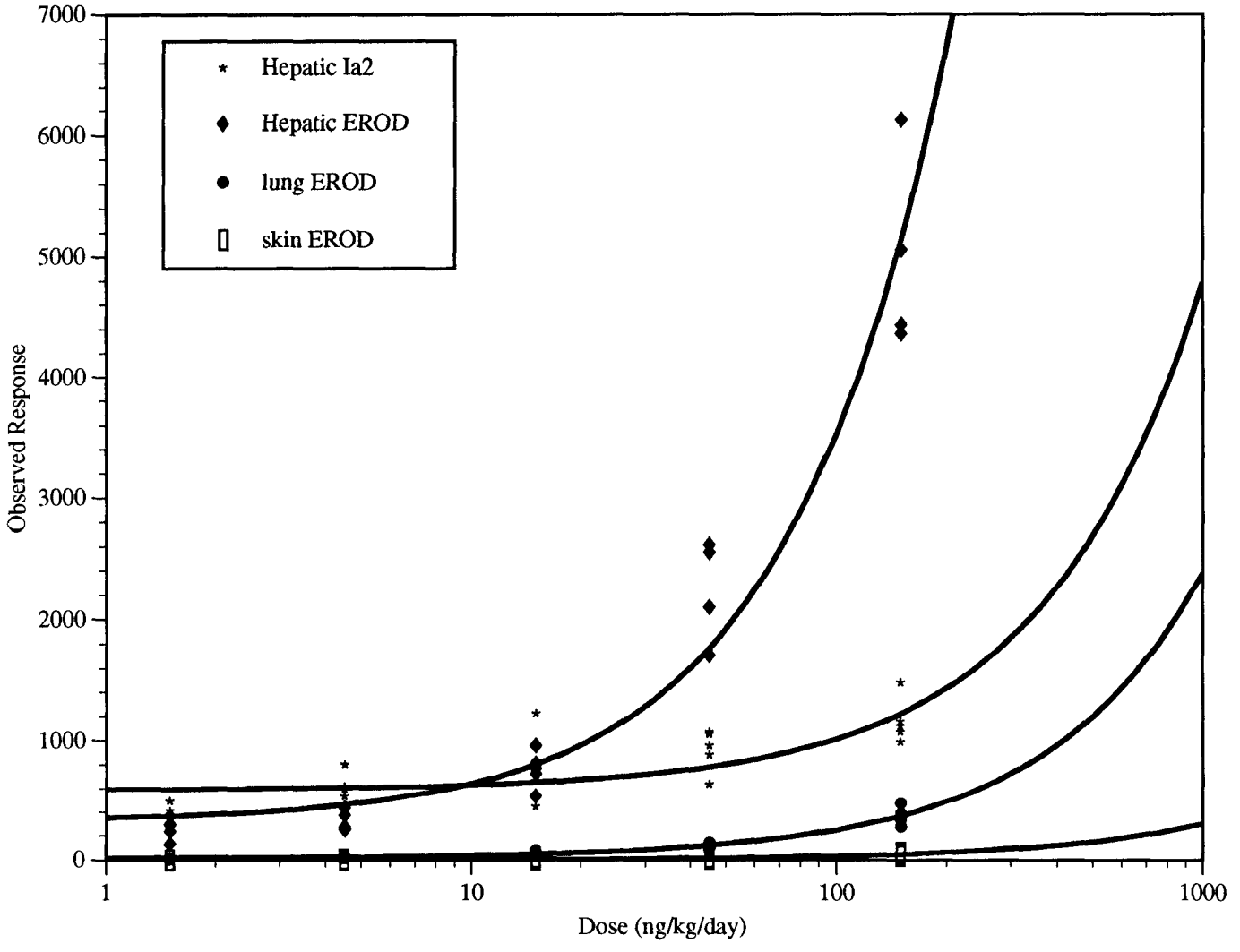
(Graph in Log-Linear Coordinates)

for

Female B6C3F1 Mice

(DeVito et al., 1992a)

Response: 13 week dosing 5 x per week % increase Hepatic EROD, Hepatic 1a2, Skin and Lung EROD



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Linear Function)

Hepatic EROD: f(x) = 4.188324E+0*x+5.909044E+2, R^2 = 5.364690E-1

Hepatic 1a2: f(x) = 3.2.205153E+1*x+3.213650E=2, R^2 = 9.458424E-1

skin EROD: f(x) =2.996198E-1*x+2.254425E+0, R^2 = 9.113437E-1

lung EROD: f(x)= 2.360102E+0*x+1.561678E+1, R^2 = 9.399031E+1

Graph P2

Dose-Response

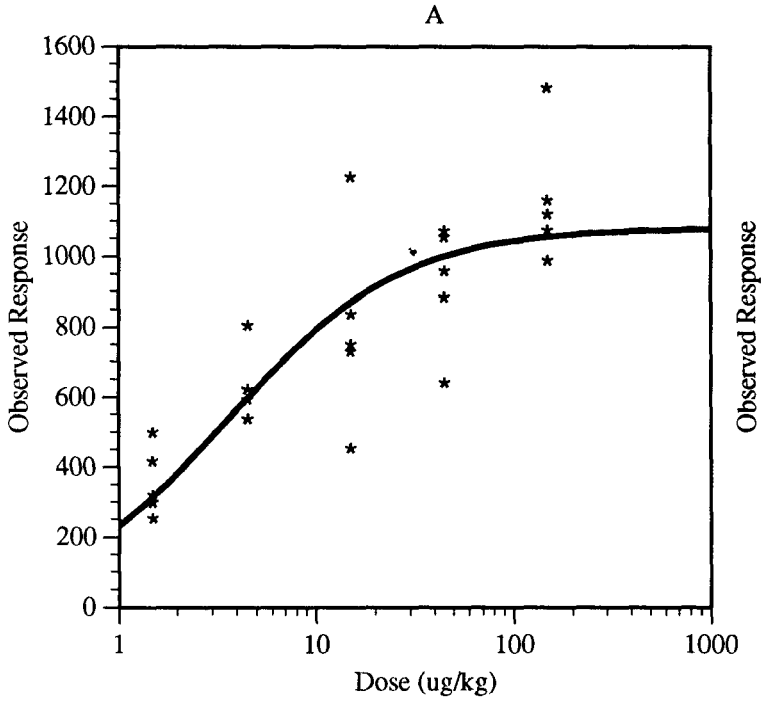
(Graph in Log-Log Coordinates)

Female B6C3F1 mice

(DeVito et al, 1992a)

Dose: 5 X weekly 13 weeks

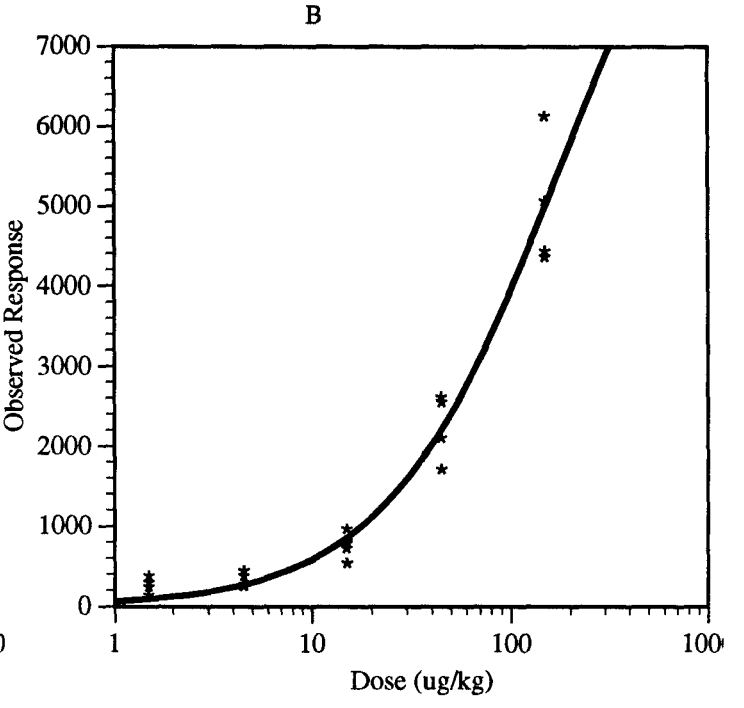
Response:% increase Cyp1A2 level, Hepatic EROD, Lung EROD and Skin EROD



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" type response

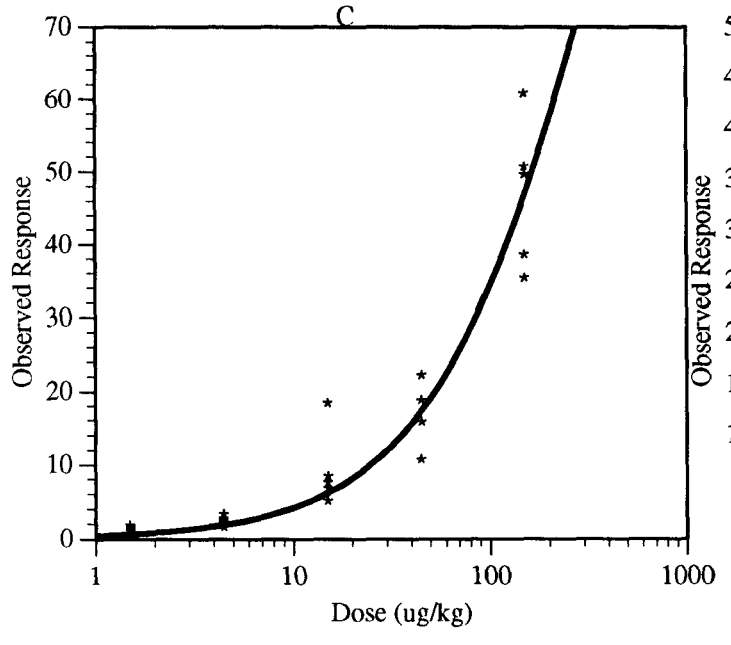
Hepatic 1A2 f(x)=1+1.080720E+3*x/(3.669929E+0+x)



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" type response

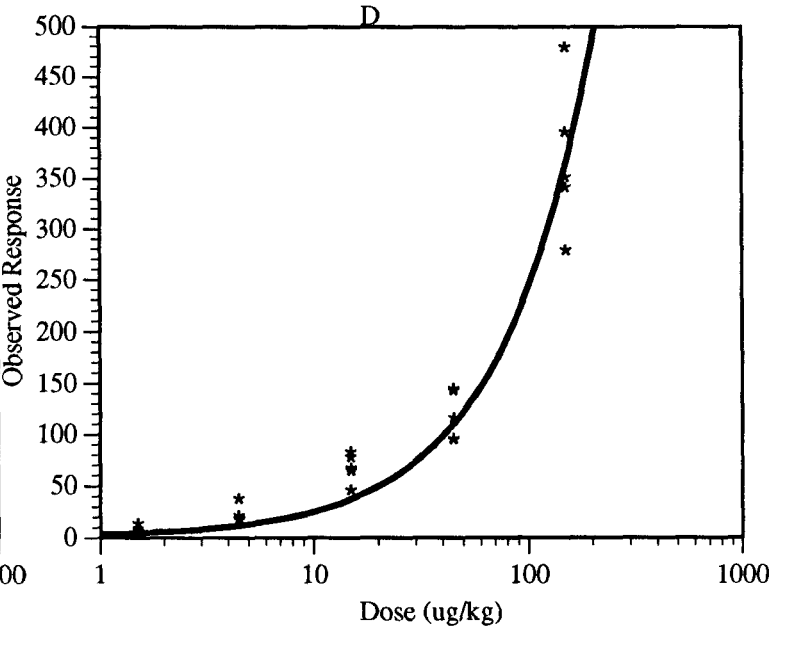
Hepatic EROD f(x)= 1.088571E+4*x/(1.766552E+2+x)



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "1Michaelis-Menten" type response)

Skin EROD f(x) = 1.734444E+2*x/(4.054377E+2+x)



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" type response)

Lung EROD f(x) = 1+-2.521625E+9*x(-1.0431179+9+x)

Graph P3

Dose-Response

(Graph in Log-Log Coordinates)

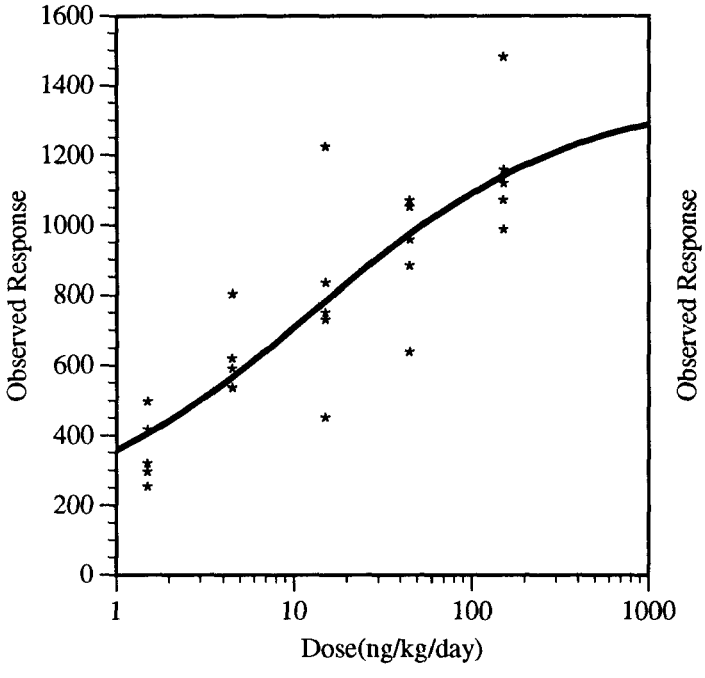
Female B6C3F1 mice

(DeVito et al, 1992a)

Dose: 5 X weekly

Response:% increase Cyp1A2 level, Hepatic EROD, Lung EROD and Skin EROD

A



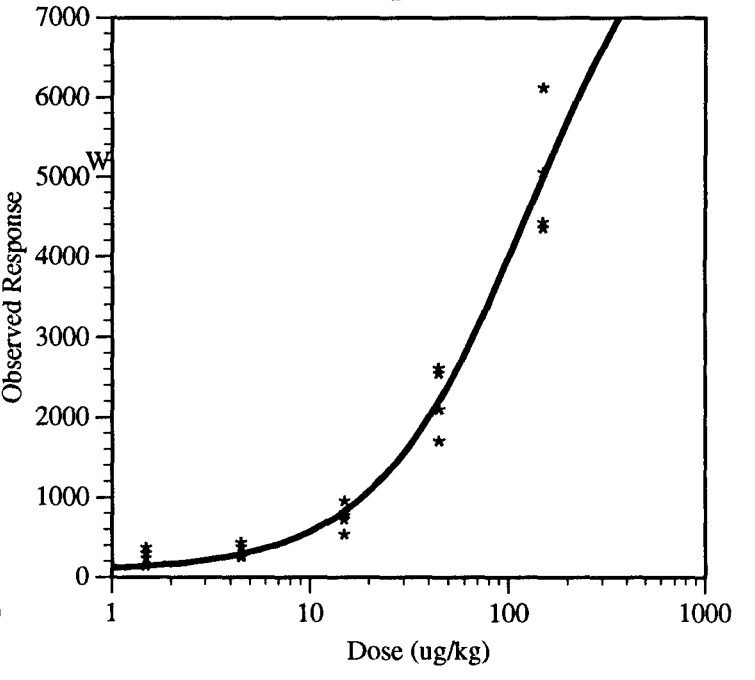
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for " Hill function + background parameter)

1A2 levels f(x) = 1.242448E+3*x^5.924782E-1) /

4.586583E+0+x^5.924782E-1) + 135

B



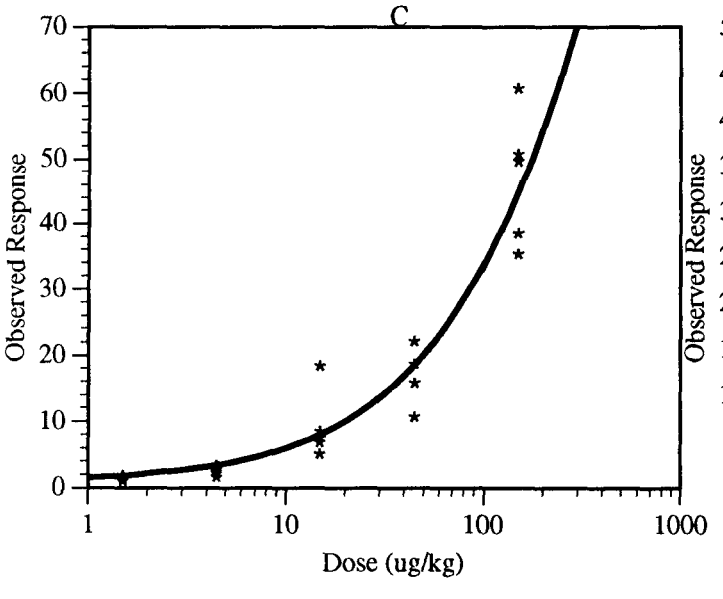
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill function" type response)

Hepatic EROD f(x) =9.115251E+3*x^1.115666E+0/

2.283763E+2+x^1.115666E+0 +80)

C



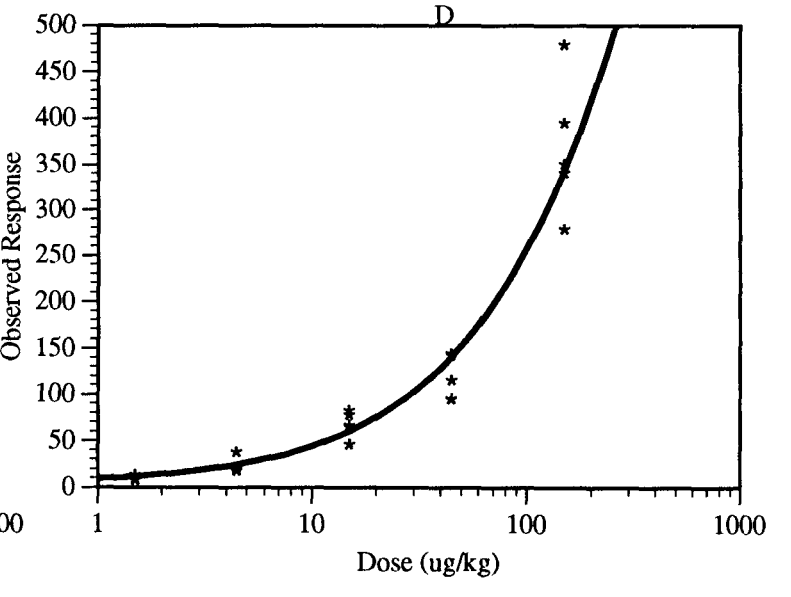
Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill Function" type response)

Skin E ROD f(x) =2.397953E+2*x^8.615018E-1

/3.332969E+2+x^8.615018E-1 +0.83

D



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Hill Function" type response)

Lung EROD f(x) =2.070050E+3*x^8.436144E-1/

(3.480857E+2+x^8.436144E-1) +3.19

Graph P4

Comparative Dose-Response

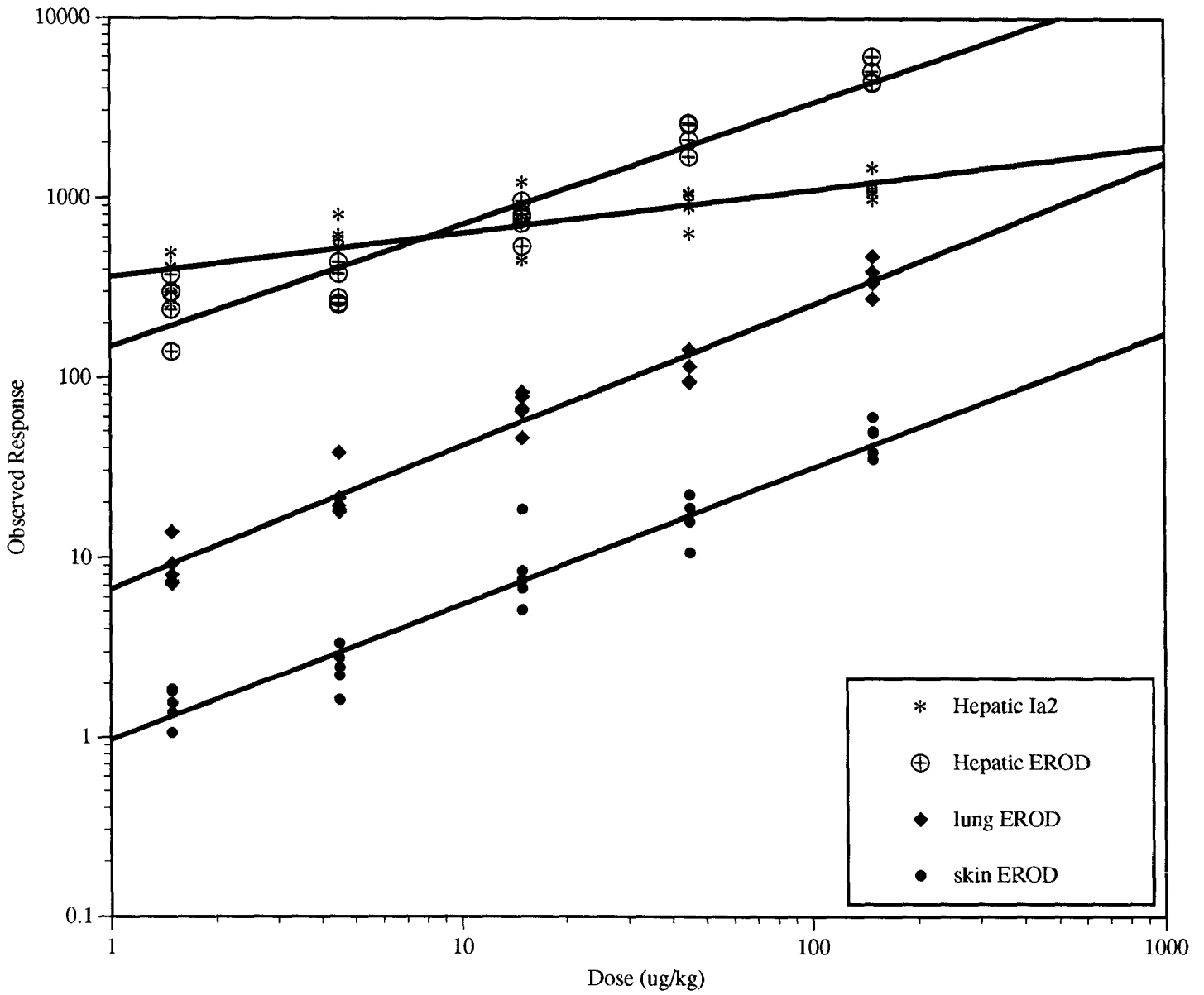
(Graph in Log-Log Coordinates)

Female B6C3F1 Mice

(DeVito et al., 1992a)

Dose: p.o. 5 X per week 13 weeks

Response: % increase Hepatic EROD,Hepatic Ia2 , Skin and Lung EROD



Functional Forms of Dose-Response Curves

(Best Fit Estimates for Power Laws)

Hepatic EROD: f(x) = 1.482129E+2 * (x^6.769077E-1), R^2 = 9.229244E-1

Hepatic Ia2: f(x) = 3.650628E+2 * (x^2.423515E-1), R^2 = 7.277352E-1

Skin EROD: f(x) = 9.676016E-1 * (x^7.538292E-1), R^2 = 9.363354E-1

Lung EROD: f(x) = 6.679660E+0 * (x^7.910685E-1), R^2 = 9.658391E-1

Graph Q

Dose-Response

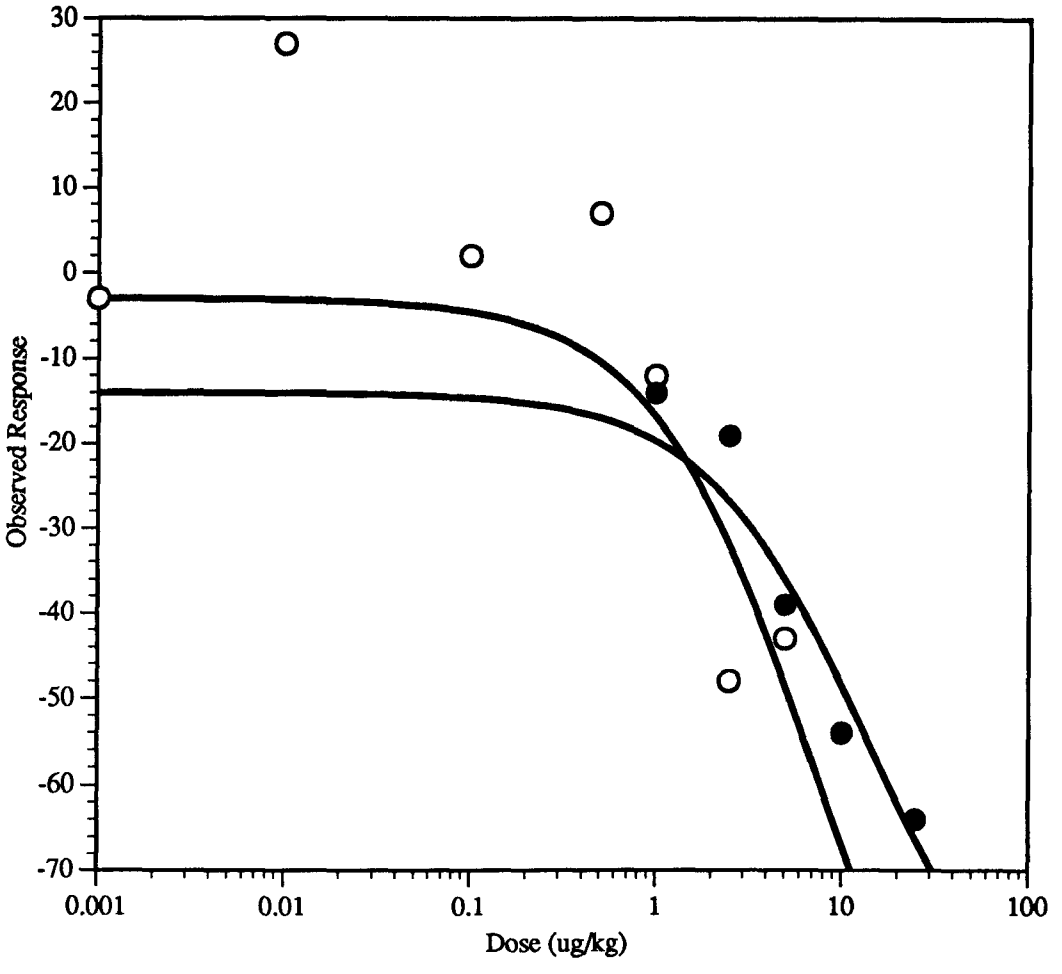
(Graph in Log-Linear Coordinates)

for

Sprague-Dawley Rat

Dose: single oral

Response: % change in liver EGR receptor binding levels



Functional Form of Sigmoid Dose-Response

(Best Fit Estimate for "Michaelis-Menten" + initial response parameter)

Intact f(x) = -14+-8.004886E+1/(1.319891E+1+x)

Adrenaalectomized f(x) = -3-1.072898E=2*x/(6.766685E+0+x)

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