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Summary of the U.S. EPA Workshop on the Relationship Between Exposure Duration and Toxicity

EPA/600/R-99/081
September 1998

SUMMARY OF THE U.S. EPA WORKSHOP ON THE RELATIONSHIP BETWEEN EXPOSURE DURATION AND TOXICITY

Sheraton Crystal City
Arlington, Virginia
August 5-6, 1998

National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC 20460

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This report was prepared by Eastern Research Group, Inc. (Contract No. 68-D5-0028) as a general report of discussions during the Workshop on Relationship Between Exposure Duration and Toxicity. As requested by EPA, this report captures the main points and highlights of discussions held during plenary sessions. The report is not a complete record of all details discussed nor does it embellish, interpret, or enlarge upon matter that were incomplete or unclear. Statements represent the individual views of each workshop participant; none of the statements represent analyses by or positions of the National Center for Environmental Assessment or the EPA.

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SECTION ONE

BACKGROUND

1.1 BACKGROUND

Current default risk assessment procedures typically define "dose" as an averaged daily exposure with an emphasis on "chronic effects" observed from long-term exposures or in chronic bioassays to characterize potential lifetime risks. However, emergency response scenarios and other regulatory implementation activities require characterizing acute exposures, and it has always been recognized that real-world exposures constitute intermittent regimens at a variety of concentrations. Contemporary toxicology and recently proposed U.S. Environmental Protection Agency (EPA) guidance are now placing emphasis on how more mechanistic data can help to inform default approaches. Toxicity can depend on not only on the magnitude but also on the duration, frequency, and timing of an exposure. Mechanistic determinants of chemical disposition (absorption, distribution, metabolism, and elimination) as well as pharmacodynamic considerations of toxicant-target tissue interaction (e.g., repair and proliferation rates) include both concentration and time-dependent processes. An "acute" exposure duration may result in "chronic" toxicity if the chemical or its damage accumulates. Thus, the choice of an appropriate measure of a "dose metric" must be defined by characterizing the exposure-dose-response continuum.

EPA's Risk Assessment Forum (RAF) is beginning to examine how dose-duration relationships are or can be incorporated into the risk assessment process for less-than-lifetime exposures. This is an extension of efforts within EPA, as well as collaborative work carried out with researchers from the Harvard School of Public Health. As part of this effort, a workshop was held on August 5 and 6, 1998, in Arlington, Virginia. The objective of the workshop was to discuss our current understanding of dose-duration relationships and their underlying mechanistic basis, the approaches that can be used in modeling these relationships, their inclusion in risk assessment, and future directions in this area. Appendix A presents the meeting agenda and Appendix B presents the charge to the participants.

1.2 THE AUGUST 1998 WORKSHOP

The RAF invited scientists with expertise in toxicology, biostatistics, and risk assessment, and epidemiology from both within and outside the Agency, to participate in the workshop (Appendix C). The workshop was designed to identify and to discuss areas of common understanding, as well as areas of differences. Prior to the workshop, each invited participant received an issues paper (Appendix D) intended to explore issues in the assessment of dose-duration effects in order to identify where the current risk assessment approach may be improved and to identify gaps in our knowledge and methodology in order to suggest areas of further research. (The paper included in Appendix D is the original draft distributed prior to the workshop; it may be revised in the future to reflect comments provided by the participants.) During the workshop, plenary presentations provided specific examples of the various issues that are defined in the paper.

Louise Ryan, Professor of Biostatistics at Harvard University's School of Public Health and Dana Farber Cancer Institute, served as the workshop facilitator. The workshop was structured as a series of alternating plenary sessions and breakout group discussions. Each participant was assigned to one of three breakout

groups. In making the group assignments, EPA sought to ensure a mixture of expertise and Agency representation in each group. Breakout group participants are listed in Sections 8.1, 8.2, and 8.3. A group leader helped to facilitate discussions in each group and a rapporteur captured key discussion points for the report back to the plenary sessions. The agenda included specific times for workshop observers to contribute comments and questions. The final workshop session addressed future directions for work on this issue over the next 5 years and beyond.

SECTION TWO

OPENING PLENARY SESSION

2.1 INTRODUCTORY PRESENTATIONS

To open the workshop, Louise Ryan, Professor of Biostatistics at Harvard University's School of Public Health, welcomed the participants and observers. She explained the purpose of the meeting and EPA's charge to the participants. Each of the invited experts then introduced themselves briefly, indicating their affiliations and describing their interest in the topic. The experts included:

William Boyes
Chief, Neurophysiological Toxicology Branch
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency

Harvey Clewell
Senior Project Manager
K.S. Crump Division
ICF Kaiser, International

Rory Conolly
Senior Scientist
Chemical Industry Institute of Toxicology

Dan Costa
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency

Dale Hattis
Research Professor
Center for Technology, Environment, and Development
Clark University

Annie Jarabek
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Gary Kimmel
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Allen Lefohn
President
ASL & Associates

James McDougal
Senior Scientist, Geo-Centers, Inc.
Operational Toxicology Branch
AF Research Laboratory

Resha Putzrath
Principal
Georgetown Risk Group

Stephen Rappaport
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School of Public Health
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Lorenz Rhomberg
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Harvard University
Center for Risk Analysis

Harvey Richmond
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U.S. Environment Protection Agency

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Louise Ryan
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Harvard University
School of Public Health

Paige Williams
Associate Professor of Biostatistics
Harvard University
School of Public Health

Ronald Wyzga
Senior Program Manager
Health Studies Program
Electric Power Research Institute

Appendix C contains the lists of contact information for invited and EPA participants, as well as biographies of the invited participants.

2.2 C × T: HISTORICAL PERSPECTIVES, CURRENT ISSUES, AND APPROACHES

Annie Jarabek, EPA National Center for Environmental Assessment

The purpose of Dr. Jarabek's presentation was to:

- Introduce the risk assessment context
- Clarify terminology
- Review assumptions in current approaches
- Identify conceptual commonalities and differences
- Engender interdisciplinary dialogue to help improve applications

2.2.1 The Risk Assessment Context

It must first be appreciated that the topic of exposure-dose-response is embedded in an overall scheme of regulatory risk assessment and management. This scheme includes inherent aspects of duration, notably assumptions in characterization that must be informed by data all along its cycle, from source characterization to transport and transformation description as it is used to define air (or water or soil) quality, which is then evaluated by exposure-dose-response assessment. This assessment is then used to inform health or ecological risk estimates that form the basis of regulatory standards that in turn dictate control technologies on the source. The intent of this workshop is to focus on the dose-response assessment aspect according to the 1983 National Academy of Sciences scheme for risk assessment versus risk management, but the broader context must be kept in mind when discussing how this information might be communicated or used in other arenas.

The concept of scale provides a framework for both human and ecological risk assessment, since spatial and temporal aspects are unifying concepts. Scale includes implicit issues of time as it relates to spatial distribution, the development or degree of severity, and when observation takes place.

A number of important exposure issues are raised when attempting to interpret the exposure-dose-response continuum. It is important to accurately characterize activity patterns (how long a person stays in an activity, where a person is relative to a certain concentration profile). Measurements must be defined with respect to time (e.g., as a daily or annual average) in order to characterize exposures. Spatial representativeness must also be considered (personal or area samples and where the sample is relative to exposure). It is also important to consider variability in measuring concentration and time when characterizing exposures.

Underlying assumptions in risk assessment and risk management should be explored. The assumptions may differ when the objective is dose-response assessment versus risk management and they typically dictate derivation approaches. In particular, we should examine underlying assumptions with respect to the exposure scenario, the effect severity, the population to be considered, the database utilized, the use of safety versus uncertainty factors, and the use of dosimetry adjustments.

Currently, exposure scenarios (acute or chronic) direct risk characterization without regard to its interface with toxicology or health outcome characterizations. Acute exposures that require regulation include emergency releases or intermittent start-up/shut-down processes, periodic contaminations, or occupational exposures. Chronic exposures are defined as "lifetime ambient," typically 70 years (with exposures or consumptions of 24 hr/day or 2 L/day). Types of toxicity data used to address these exposure scenarios include:

- Acute: 1- to 24-hour exposures, single or few oral administrations, up to 14-day exposures
- Chronic: 90-day studies, 2-year bioassays

However, these categories are based on chronologic time with no consideration of the toxicity mechanisms or appropriate ways to express the dose metric.

Default equations for duration adjustment of exposure are shown in Figure 2-1 (with the exception that in current practice these do not apply to exposures associated with developmental toxicity). The default duration adjustments assume that the internal dose is equivalent to exposure concentration. Further, it is also assumed that toxicity is related linearly to the C × T product so that equivalent C × T products are predicted to cause the same toxicity. The basis for these default equations is in Haber's Law.

2.2.2 Haber's Law

According to this "law," a constant, in this case a fixed effect level (i.e., a constant severity or incidence of a health endpoint), is related to exposure concentration and duration by the equation shown in Figure 2-2. The relationship is described by the hyperbola and the arms converge asymptotically toward the axes of the time and concentration coordinates. Because Haber examined only extremely short durations at relatively high concentrations of irritant gases, a C × T relationship appeared to hold because concentration was the dominant determinant of the observed toxicity in that limited time window. Haber's Law was extended to characterization of long-term exposures on the basis of considerations of potential accumulation of the chemical or its damage so that the toxicity observed after chronic exposures was again thought to possibly be due to the C × T product. However, Figure 2-3 illustrates how the use of this relationship might give erroneous estimates when extrapolating from an 8-hr exposure effect level to shorter and longer durations. If a single 8-hr experimental concentration is used as the basis of a C × T product calculation to estimate a 24-hr equivalent level, then the use of Haber's law results in an estimate that is conservative (presumably

Default Duration Adjustments for Noncancer Toxicity

Acute (Defined as $\leq$ 24 hours)

$$EL_{ADJ}\ (ppm) = EL\ \ (ppm)\ \ x\ \ \frac{\#\ of\ hours}{24\ hours}$$

Chronic

$$EL_{ADJ}\ (ppm) = EL\ \ (ppm)\ \ x\ \ \frac{\#\ of\ hours}{24\ hours}\ \ x\ \ \frac{\#\ of\ days}{7\ days}$$

Figure 2-1

Schematic of Relationship Between Exposure Concentration and Duration to Fixed Effect Level (EL) Assuming "Haber's Law"

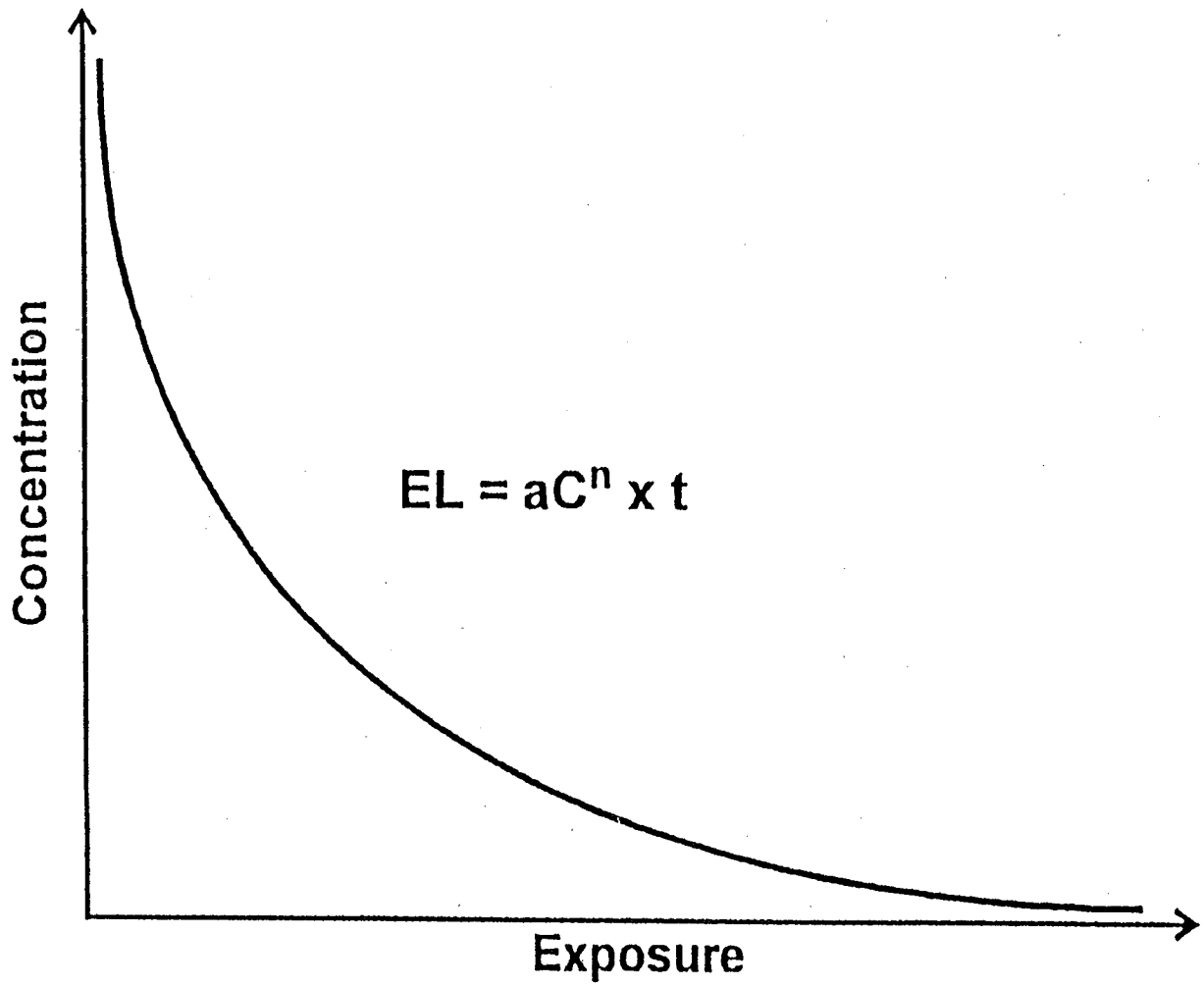


Figure 2-2

Schematic of Relationship Between Exposure Concentration and Duration to Fixed Effect Level (EL)

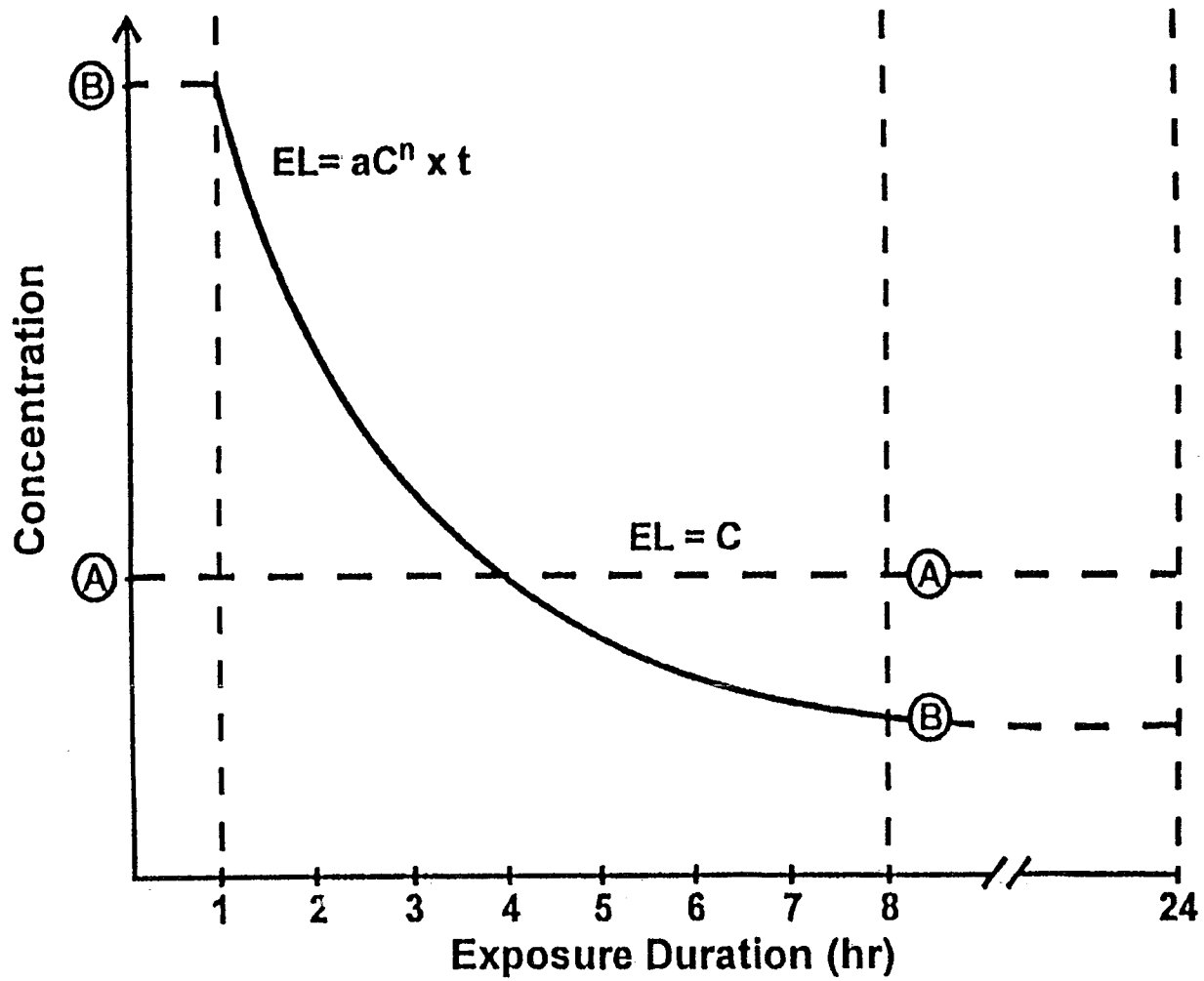


Figure 2-3

protective) relative to the one predicted if only concentration were the dominant determinant of toxicity. If the single 8-hr experimental concentration was extrapolated to a 1-hr estimate, however, assumption of Haber's law (B) results in an overestimate of the effect level when compared to an estimate assuming concentration alone (A) was the determinant.

The problem with this default, as with any other, is that it relies on a rudimentary description of a complex and dynamic system. As illustrated in Figure 2-4, toxicity depends on the magnitude, duration, and frequency of exposure. Thus, the choice of a metric to characterize this toxicity should depend on some knowledge of the mechanism by which the toxic effects are induced. Current research in toxicology and modeling allows us to now define alternative dose metrics that begin to better embody considerations of these underlying mechanisms.

2.2.3 Dose Metrics

Alternative potential dose metrics include:

- Blood concentration of parent chemical
- Area under blood concentration curve (AUBC) of parent chemical
- Tissue concentration of parent chemical
- Area under tissue concentration curve (AUTC) of parent chemical
- Tissue concentration of metabolite
- AUTC stable metabolite
- AUTC reactive metabolite

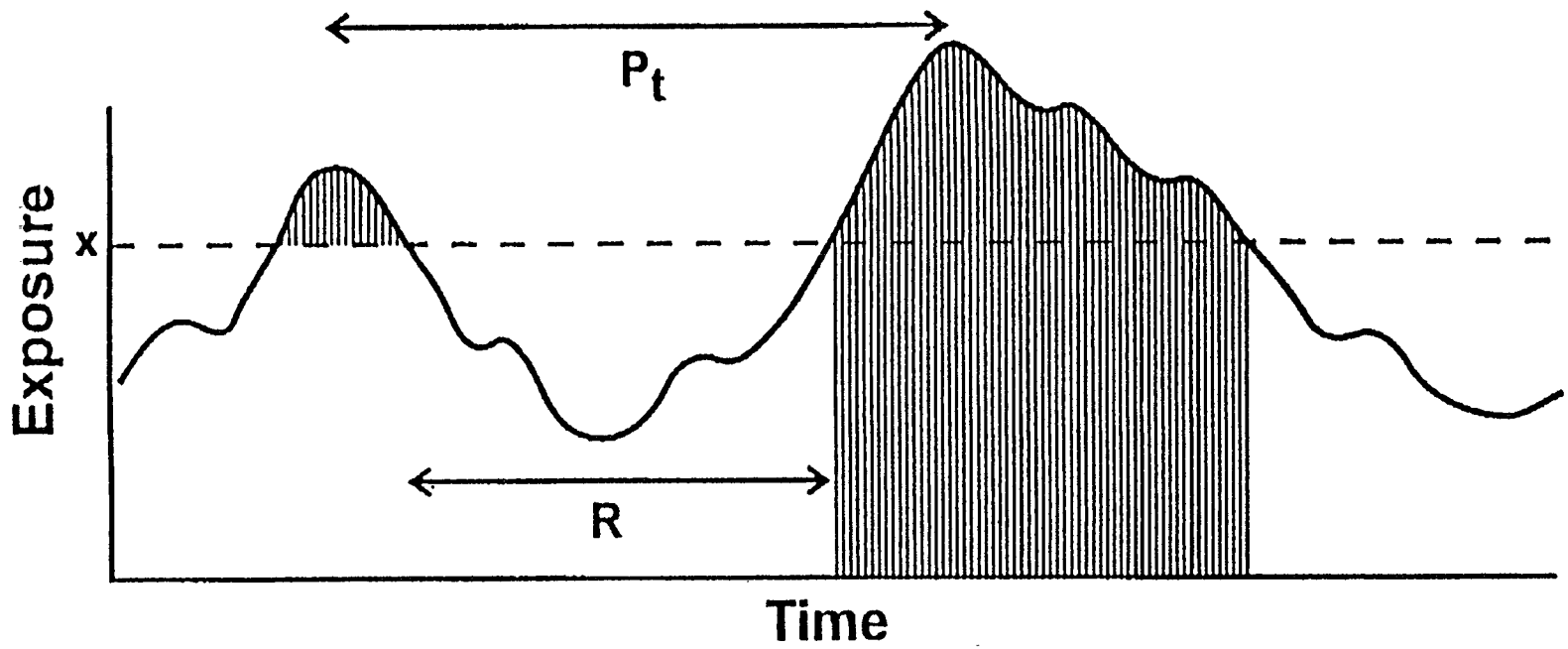
Toxicologists recognized early on that detoxication could occur and that expressing C × T relationships on the basis of exposure was inadequate. Hayes restated the C × T relationship in 1975 to address dosimetry (Figure 2-5). Likewise, the pharmaceutical industry looked at physiologic time rather than chronologic time as a way to normalize the plasma half-life of drugs across species (Figure 2-6).

For accurate risk assessments, it is ultimately desirable to have a comprehensive biologically based dose-response model that incorporates the mechanistic determinants of chemical disposition, toxicant-target interactions, and tissue responses integrated into an overall model of pathogenesis (Figure 2-7). Incorporating a comprehensive description of the exposure-dose-response continuum can allow us to be more quantitative and to move from estimates presumed to be protective to actual predictive estimates. Mechanistic models have been demonstrated to be particularly effective, particularly with respect to more accurate description of chemical disposition.

Mechanistic models can account for both time- and concentration-dependent parameters and processes, including chemical disposition (deposition, absorption, distribution, metabolism, and elimination) and endpoint (severity, window of observation).

Along the exposure-dose-response continuum, exposure factors that govern absorption include concentration, contact duration, frequency, dosing pattern, dosing vehicle, fed or fasted state of test species, occlusion, release from vehicle, and transit/residence time. Factors governing absorption also include physicochemical characteristics such as dissociation state, molecular size, molecular weight, partition coefficient, pKa, reactivity, solubility, volatility. Portal-of-entry factors that govern absorption include the barrier capacity, particularly as it is related to variability in species and individuals, blood flow rate, cell turnover, cell types

Potential Dose Profile Metrics



"Effective Exposure" depends on magnitude, duration, and frequency of exposure. Timing in turn can affect these parameters.

Figure 2-4

Hayes (1975)

- **Restated "C x t" relationship to address dosimetry:**

$$D = (CV_m - D_e)\ tr/w$$

Where:

D = dosage (mg/kg) in time (min);

C = concentration (mg/m^3);

Vm = respiration minute volume (m^3/min);

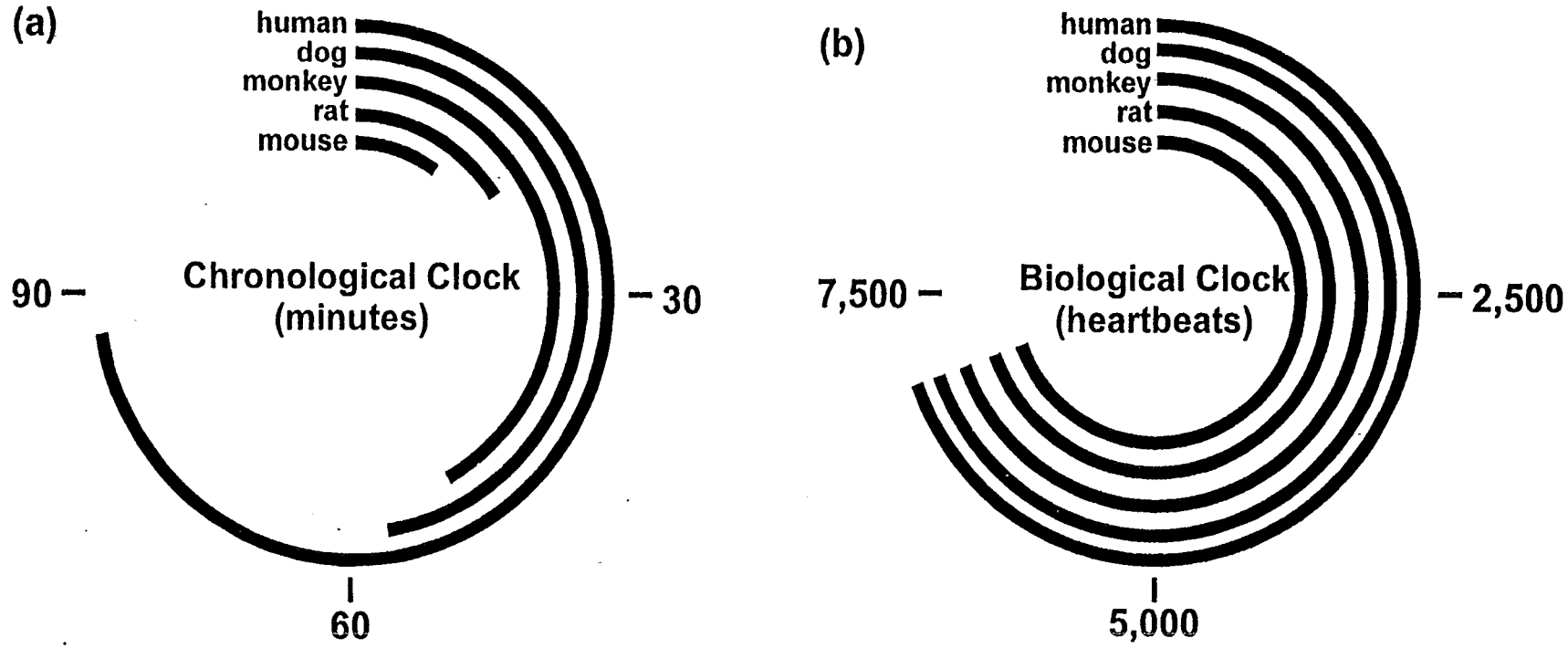
De = detoxication rate (mg/min);

R = retention coefficient; and

w = body weight

Figure 2-5

Chronologic vs. Physiologic TIme



Ceftizoxine half-life in various mammals, expressed either in chronological time in min (a), or physiologic time, in heartbeats (b).

Figure 2-6

(Mordenti, 1985)

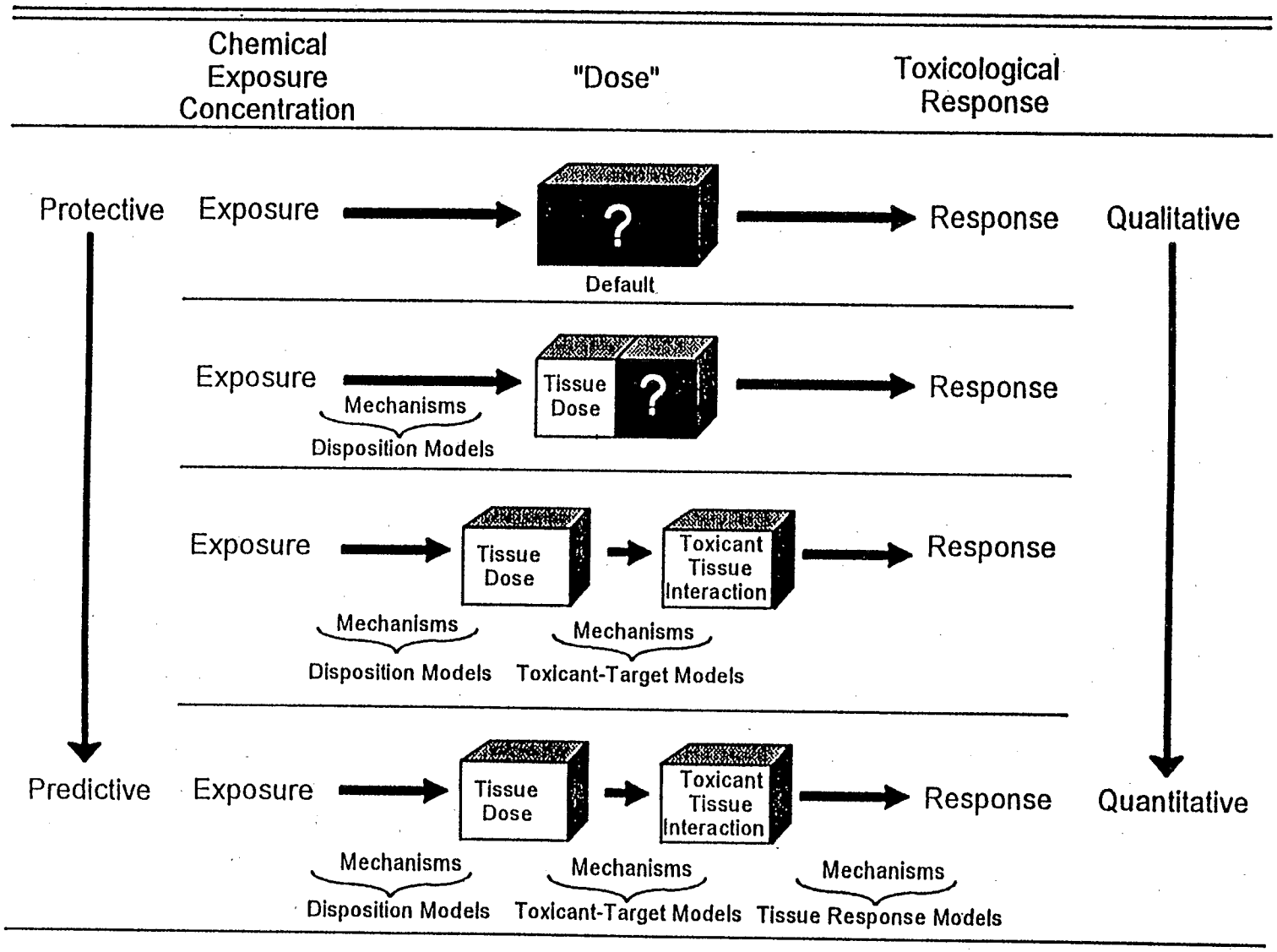

Chemical Exposure Concentration
"Dose"
Toxicological Response
Protective
Predictive
Qualitative
Quantitative
Exposure
?
Default
Response
Exposure
Mechanisms
Disposition Models
Tissue Dose
?
Response
Exposure
Tissue Dose
Toxicant Tissue Interaction
Response
Mechanisms
Disposition Models
Mechanisms
Toxicant-Target Models
Exposure
Tissue Dose
Toxicant Tissue Interaction
Response
Mechanisms
Disposition Models
Mechanisms
Toxicant-Target Models
Mechanisms
Tissue Response Models

Figure 2-7

and morphology, contact site, contact area, contact duration, diffusion to blood, intactness of organ, metabolism, pH of portal of entry, recirculation, specialized absorption sites, and storage in cells. Factors governing target tissue dose include metabolism/clearance rates, tissue binding, tissue blood flows, tissue/blood partition coefficients, and tissue coefficients. Many of these aspects will be discussed in the following plenary presentations.

It is important to remember that mechanistic inferencing is a key consideration for the choice of dose metric and to describe it when developing any dosimetry or PBPK model. For example, the model structure and metric will be dictated by the effect of irritants on the portal of entry, and the volume of distribution and half-life of a chemical with remote toxicity.

EPA now uses some form of mechanistic descriptions of dosimetry in its reference concentration methods and this is being adopted to other approaches in the Agency. It must be remembered that these approaches are applied to a database of information on the effects of a chemical and that the objective is to evaluate the potential for a chemical to induce toxicity at any of the critical life stages from conception through geriatrics, including development and reproduction. This translates into data requirements. For example, the minimum database for derivation of an RfC includes two chronic inhalation bioassays in two different species, one two-generation reproductive study, and two developmental toxicity studies in two different species. These will capture toxicity across the life span and species sensitivity. If interaction only occurs with the respiratory tract, the two-generation or developmental study may not be necessary. Uncertainty in the estimate results if these data are not available and there are no mechanistic data by which to better understand the mechanisms of potential toxicity. Consideration of this uncertainty has an important historical component that may be useful to the workshop.

2.2.4 Historical Perspective on Dose-Response Assessment

Historically, risk assessment procedures focused on uncertainty because of limitations in experimental design, measurement techniques and animal models. Because of the limited understanding of underlying mechanisms, separate approaches for cancer and noncancer endpoints were developed. But contemporary toxicology is much more informative. Bioassays and databases are more comprehensive. Measurements are more sophisticated. There is a growing understanding of mechanisms at the molecular level. Animal models of susceptibility are used, and enhanced computational capacity exists to describe processes quantitatively.

Recently, the National Research Council has emphasized the use of mechanistic data, saying that analyses will improve as we learn more about biology, chemistry, physics, and demography. That has been reflected in an emphasis on mechanistic information in the proposed cancer guidelines. In hazard assessment, not only tumor data are considered, but also the mode of action, as well as structural-activity relationships, toxicokinetic/dosimetry studies, toxicity and pathology findings, and physical chemical properties.

This hazard assessment informs the likelihood and conditions of human exposure and mode of action conclusions to help determine how one extrapolates in the dose-response assessment. Biologically based dose-response modeling is preferable; short of that, linear and non-linear default choices exist for extrapolating in the low-dose range.

An emphasis on precursor lesions and mechanistic information in the proposed EPA cancer guidelines mirrors some of the tenets in molecular epidemiology: that early biological effects are more prevalent than later events in the population at risk; that later events (disease) have been of historical interest but earlier

events may be more specific to the exposure; and that technological advances allow xenobiotics to be directly quantified in the body or indirectly measured by identification of a dose-related biologic response.

As in molecular epidemiology, construction of characterization of the exposure-dose-response continuum involves the following progression: exposure, internal dose, biologically effective dose, biological effect, altered structure or function, clinical disease, prognostic significance. A marker can be of exposure and effect or susceptibility, depending on how the dynamic relationship plays out. One-to-one sequential linkage between each biological markers does not need to exist; correlations are useful for dose-response assessment even if the mechanistic underpinnings are unknown (Figure 2-8). These linkages can be informative for risk assessment and should be explored. They are also useful to frame future research.

It is possible to extend dose-rate models by adding parameters (e.g., duration, gestational days, means of responses, severity categories). The key question is where these approaches interact with respect to levels of biological organization (i.e., biochemical/molecular, subcellular/organelle, cellular, tissue or organ, whole organism, population) and how this influences the choice of the dose metric as well as the endpoint.

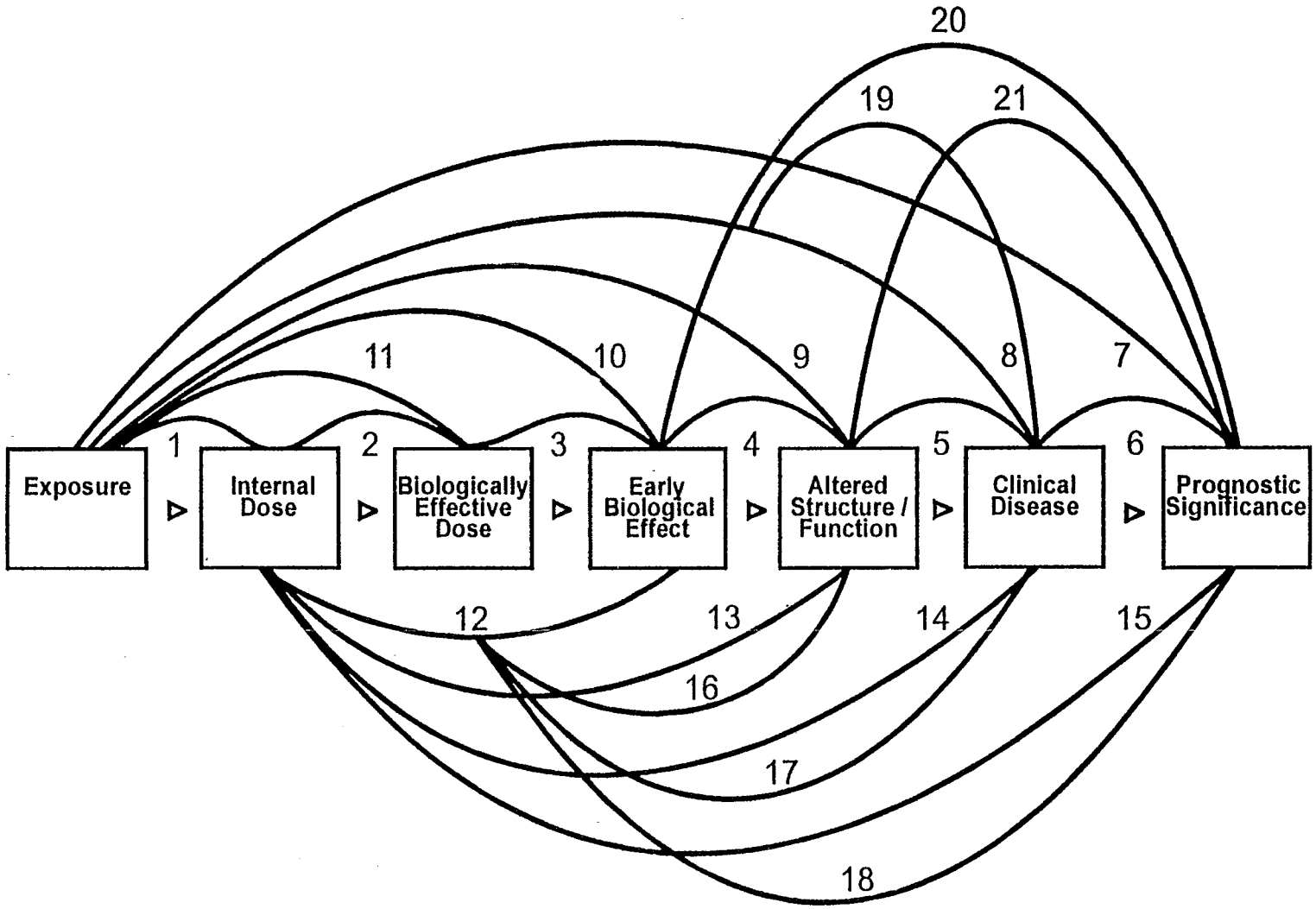
2.2.5 Harmonization of Noncancer and Cancer Endpoints

Focusing on mode-of-action information might provide a means whereby approaches to noncancer and cancer assessments begin to converge since some of the same cellular events (e.g., erosion and atrophy with subsequent cellular proliferation) may be underlying events to various lesions in common. As an example, EPA has participated in a public-private partnership with the Vinyl Acetate Task Group to look at how mechanistic information might harmonize cancer and noncancer approaches and change the dose metric for the inhalation risk assessment for vinyl acetate.

In a 2-year bioassay for vinyl acetate, tumors were observed only at last sacrifice at highest concentration (600 ppm); "noncancer" toxicity endpoints included olfactory degeneration, basal cell hyperplasia (strong dose-response above 50 ppm, sometimes at interim sacrifices). Metabolism by carboxylesterase of vinyl acetate to acetaldehyde and acetic acid is thought to be the underlying mechanism of its cytotoxicity.

The default dosimetry model for a Category 1 gas, one that interacts intimately with the respiratory tract, describes parent uptake in the entire upper respiratory tract region as a function of the overall mass transfer coefficient. Although species-specific, this mass transfer approach currently does not provide localized description of dose to, for example, in this case, the olfactory tissues. The default model also does not provide for description of other dose metrics such as the amount of acid formed or the resultant change in intracellular pH. A PBPK model was developed by researchers at DuPont Haskell laboratory that describes species-specific airflow and then metabolism in the olfactory tissue compartments. This model allowed full description of the exposure-dose-response continuum for vinyl acetate as shown in Figure 2-9, and shows the relationship between the "noncancer" cytotoxic events and subsequent tumor formation. The dose-response can now be constructed using alternative dose metrics such as tissue concentration of acid and the biological responses can include loss of pH control or some other precursor lesion such as degeneration or cell proliferation. Development of such a model is consistent with the Agency's hierarchy scheme shown in Figure 2-10. The models differ in their ability to characterize dominant determinants of the disposition (e.g., localized versus regional compartment; mass transfer versus tissue concentration; parent versus metabolite) and endpoint. These more comprehensive descriptions must be accommodated in how uncertainty factors are currently applied with a change to consideration of the degree of confidence in the description to capture critical events along the exposure-dose-response continuum.

Representation of Possible Relationships to Research



Source: Schulte (1989)

Figure 2-8

Biological Marker Components in Sequential Progression Between Exposure and Disease

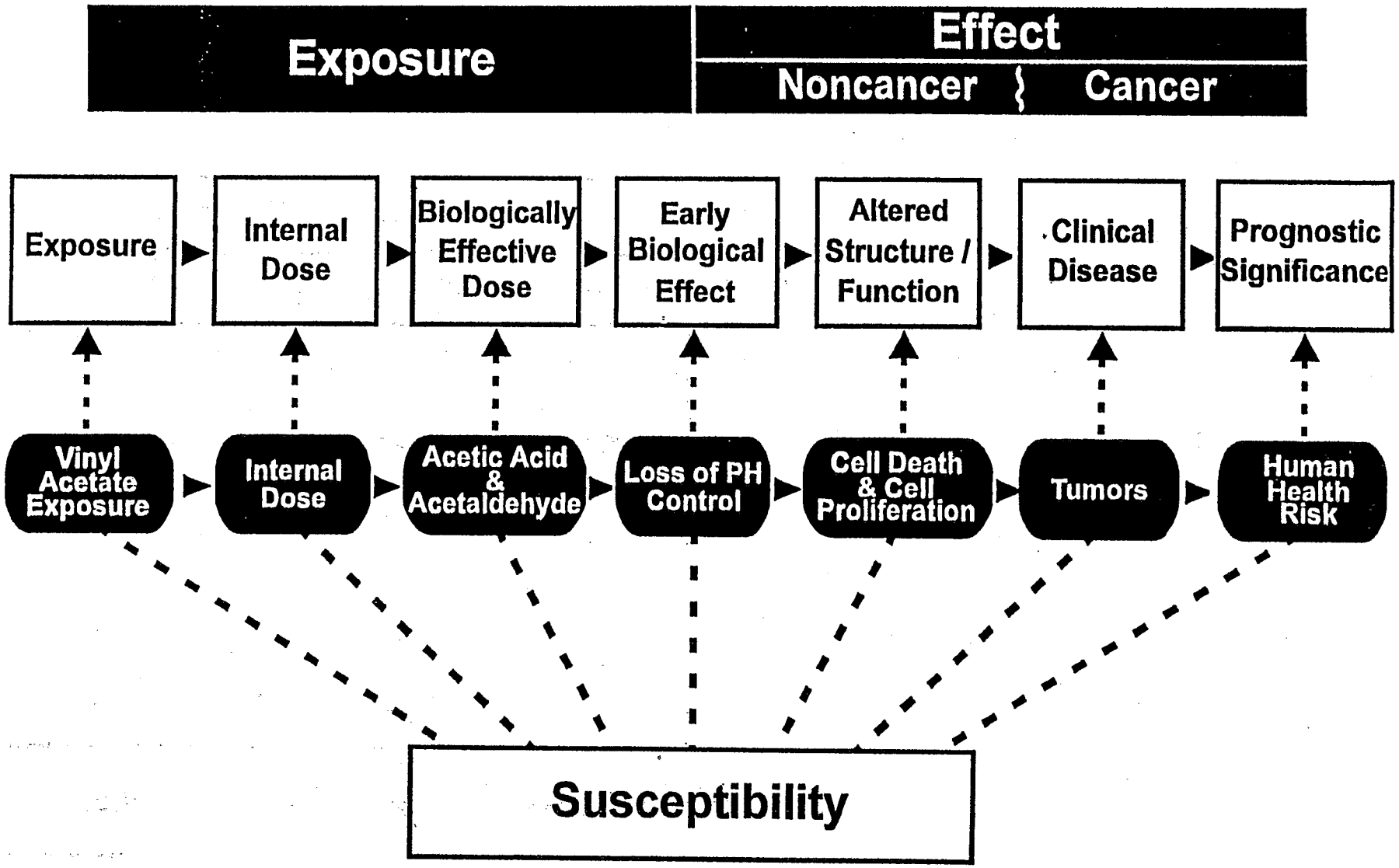


Figure 2-9

Hierarchy of Model Structures for Exposure-Dose-Response and Interspecies Extrapolation

"Optimal" model structure

- Structure describes all significant mechanistic determinants of chemical disposition, toxicant-target interaction, and tissue response
- Uses chemical-specific and species-specific parameters
- Dose metric(s) described at level of detail commensurate to toxicity data

Default model structure

- Limited or default description of mechanistic determinants of chemical disposition, toxicant-target interaction, and tissue response
- Uses categorical or default values for chemical and species parameters
- Dose metric(s) at generic level of detail

U.S. EPA (1994)

Figure 2-10

2.2.6 Variability and Uncertainty

Variability (heterogeneity in time and space) and uncertainty (lack of knowledge) are important to consider for human health dose-response assessment. Currently, uncertainty factors are not mechanistically motivated; they are derived looking across chemicals and across species. One of the challenges is to see how mechanistic modeling might improve the framework for uncertainty analysis.

Mechanistic models explicitly identify sources of variability and uncertainty (e.g., airflow patterns, partition coefficients, and tissue-specific clearance rates) and illuminate data gaps. Model validation reduces uncertainty about processes governing toxicokinetics.

EPA is developing mode of action motivated dosimetry. This includes approaches for inhalation, oral, and dermal uptake and disposition. It addresses toxicity (i.e., harmonizes "noncancer" and "cancer" approaches). It includes structures to address temporal and spatial considerations. It provides a framework for considering mechanistic data and uncertainty more explicitly.

Updating risk assessment with the state of the science will take time, and will involve the following:

- Expanding the role of mechanistic information
- Emphasizing full characterization
- Developing more sophisticated (semi-empirical and mechanistic) models for dose-response analysis
- Acknowledging a lag time before the new science is accepted in regulatory application
- Enhancing computational capacity to describe processes quantitatively

The challenge is that the quality of a prediction is a function of several things: the data, an understanding of the biology, the modeling assumptions, and the modeling methods.

2.2.7 Discussion Questions

Key discussion issues to address this framework include the following:

- What biological level can or should be assessed in establishing a toxic exposure?
- Is it necessary to have an understanding of the mechanism of toxicity?
- How do such tissue and cellular functions as metabolism, repair, and bioaccumulation factor into defining the relationship?
- Is it possible to adequately describe a concentration-duration relationship without measuring effects at biologic levels below the whole animal-systemic?

- Many of the guidelines reflecting the endpoints in Table 1 of the discussion paper (Appendix D, page 7) are based on exposures of different durations than the guideline, and have used Haber's law for extrapolation purposes. Are these guidelines reliable? How can development of these guidelines be improved?

- Are the models developed for assessing the joint effects of exposure level and duration reliable enough to allow extrapolation to doses not considered in fitting the models? How can these models be verified? How can we summarize uncertainty when extrapolating a model beyond the range of duration included in the data to which it was fit?

- How can mechanistic information and exposure metrics derived from PBPK models be better incorporated into models that evaluate dose-rate effects?

- How can study designs be improved to use information from previous exposures within the same or other studies to determine the most efficient subsequent exposure?

- What is the appropriate metric to use for reflecting exposure levels and durations in dose-rate studies? How can the type of endpoint and the mechanism of action be utilized to choose the appropriate metric?

- What are the most important areas that require strengthening in the assessment of dose-rate effects?

SECTION THREE

PLENARY PRESENTATIONS: ENDPOINTS OF TOXICITY

3.1 DEVELOPMENTAL TOXICITY: THE EFFECTS OF TEMPERATURE AND EXPOSURE ON IN VITRO DEVELOPMENT AND RESPONSE-SURFACE MODELING OF THEIR INTERACTION

Gary Kimmel, EPA National Center for Environmental Assessment

Dr. Kimmel described a study carried out in collaboration with Harvard University School of Public Health Department of Biostatistics and the U.S. Food and Drug Administration's Center for Devices and Radiologic Health. The environmental exposure used to study C × T was hyperthermia. Effects were observed that could not be accounted for only by the temperature, so duration of exposure was examined as well. This work relates to a very specific aspect of developmental toxicity within a limited range of what is considered developmental toxicity. Developmental toxicity is not a system-related event, but a time of exposure-related event. Many endpoints in development are examined.

The in vitro model is a useful tool for looking at C × T relationships in development. The embryo is taken out of the mother, isolating the individual unit, taking it out of any metabolic system, and allowing exposure of the embryo to clear "blocks" of exposure (i.e., raising temperature within 2 minutes to any level and lowering it back to the control level).

In this experimental design, embryos are explanted, equilibrated at 37° C, allowed to develop over 4 hours, then exposed to a variety of temperatures (up to 42.0) and exposure time (up to 60 minutes). They are then cultured overnight at 37° C and evaluated for viability, growth, and morphology. Only at a discrete period of development (day 10 of gestation in the rat) is considered. Table 3-1 shows the number of embryos cultured in each temperature-duration combination.

Moving from the more extensive to more subtle exposures, it is important to consider whether data can best be obtained by using a balanced design, in which all combinations have an identical number of animals, or by not using a balanced design.

The percent of affected animals (showing changes in growth, morphology, or viability) is shown in Table 3-2. The data show a relationship between effects and the combination of temperature and duration. Figure 3-1 is a plot based on Haber's law to constrict the model of these data—an equal response for any similar combination of the C × T multiple. Figure 3-2 is an expanded model, looking not only at the combination of concentration and times, but the additional effect of time or concentration individually on the model. It shows that shorter durations at higher concentrations (temperatures) produce greater effects than comparable lower temperatures at longer durations of exposure. The same relationship holds for non-viability (Figure 3-3). Growth parameters and morphologic parameters examined generally showed the same effect.

Objectives addressed in the study included:

- To develop dose-response models that reflect the interrelationship between exposure intensity, duration, and effect, incorporating multiple outcomes.

Table 3-1

Experimental Design: Number of embryos cultured in each temperature-duration combination.

	Mins 5	10	15	20	30	45	60
°C							
37.0	11	12	12	13	12	19	12
40.0	12	11	12	10	11	10	12
40.5	10	10	10	11	13	10	10
41.0	11	10	11	12	12	10	0
41.5	10	10	10	10	12	11	0
42.0	10	10	10	10	10	10	0

Table 3-2

Percent of embryos "affected" at each temperature-duration combination.							
Mins	5	10	15	20	30	45	60
°C							
37.0	0	8	8	31	17	11	17
40.0	17	36	33	30	46	20	68
40.5	30	50	40	55	54	50	60
41.0	36	30	46	75	50	90	------
41.5	50	50	40	40	58	82	------
42.0	40	70	80	100	90	80	------

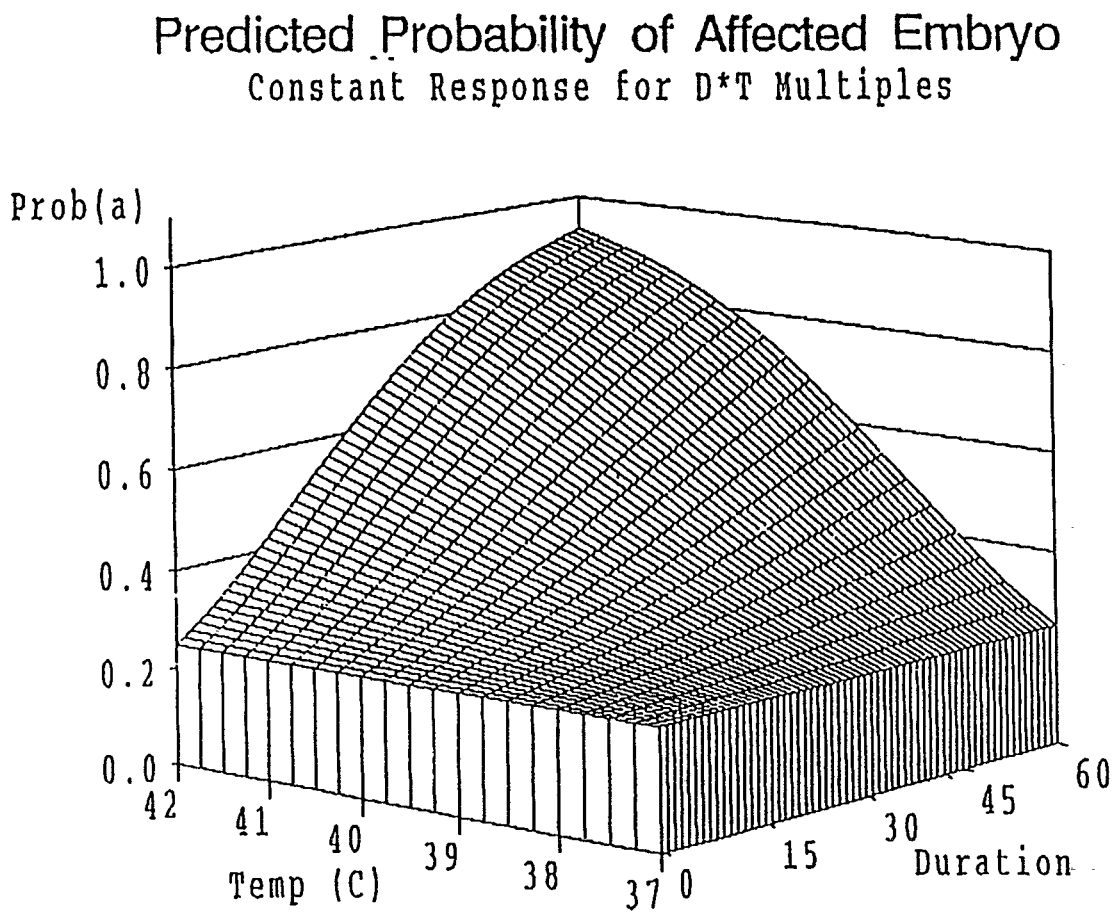

Predicted Probability of Affected Embryo
Constant Response for D*T Multiples
Prob(a)
1.0
0.8
0.6
0.4
0.2
0.0
42
41
40
39
38
37
Temp (C)
0
15
30
45
60
Duration

Figure 3-1

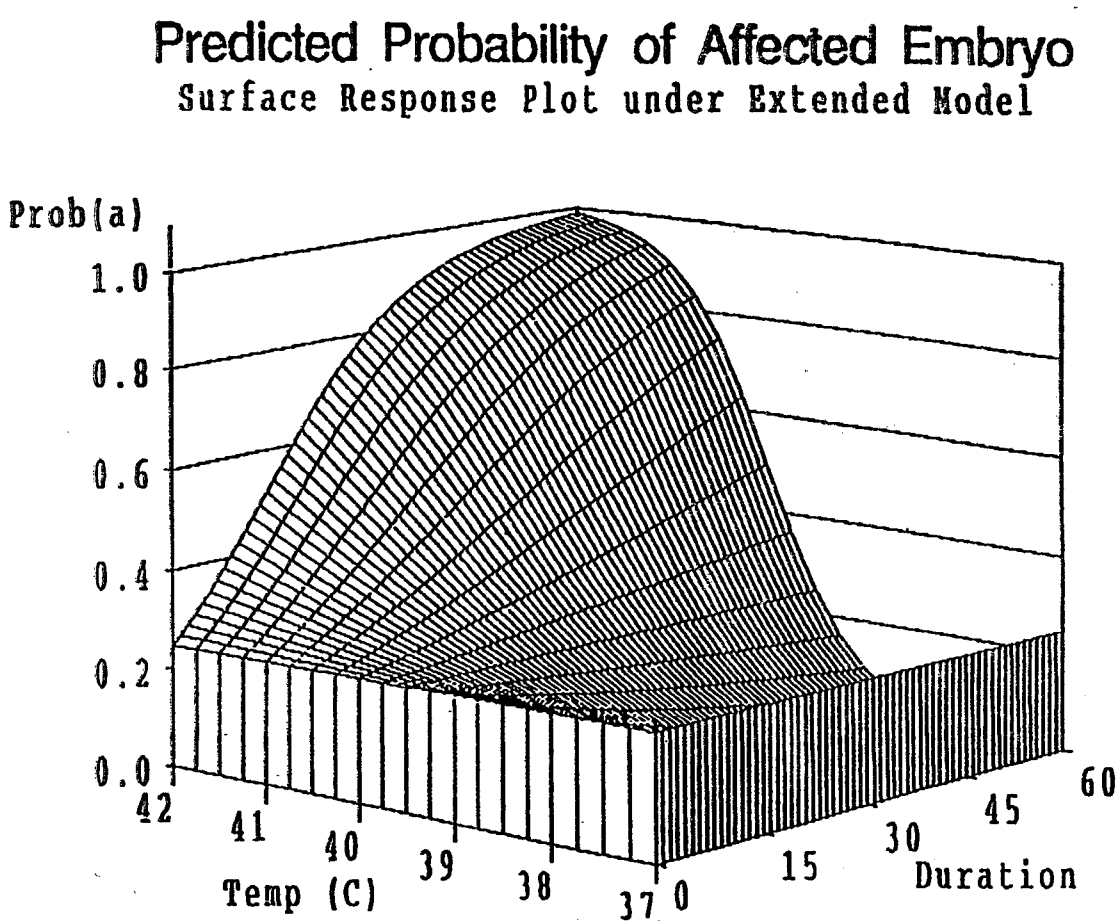

Predicted Probability of Affected Embryo
Surface Response Plot under Extended Model
Prob(a)
1.0
0.8
0.6
0.4
0.2
0.0
42
41
40
39
38
37
Temp (C)
0
15
30
45
60
Duration

Figure 3-2

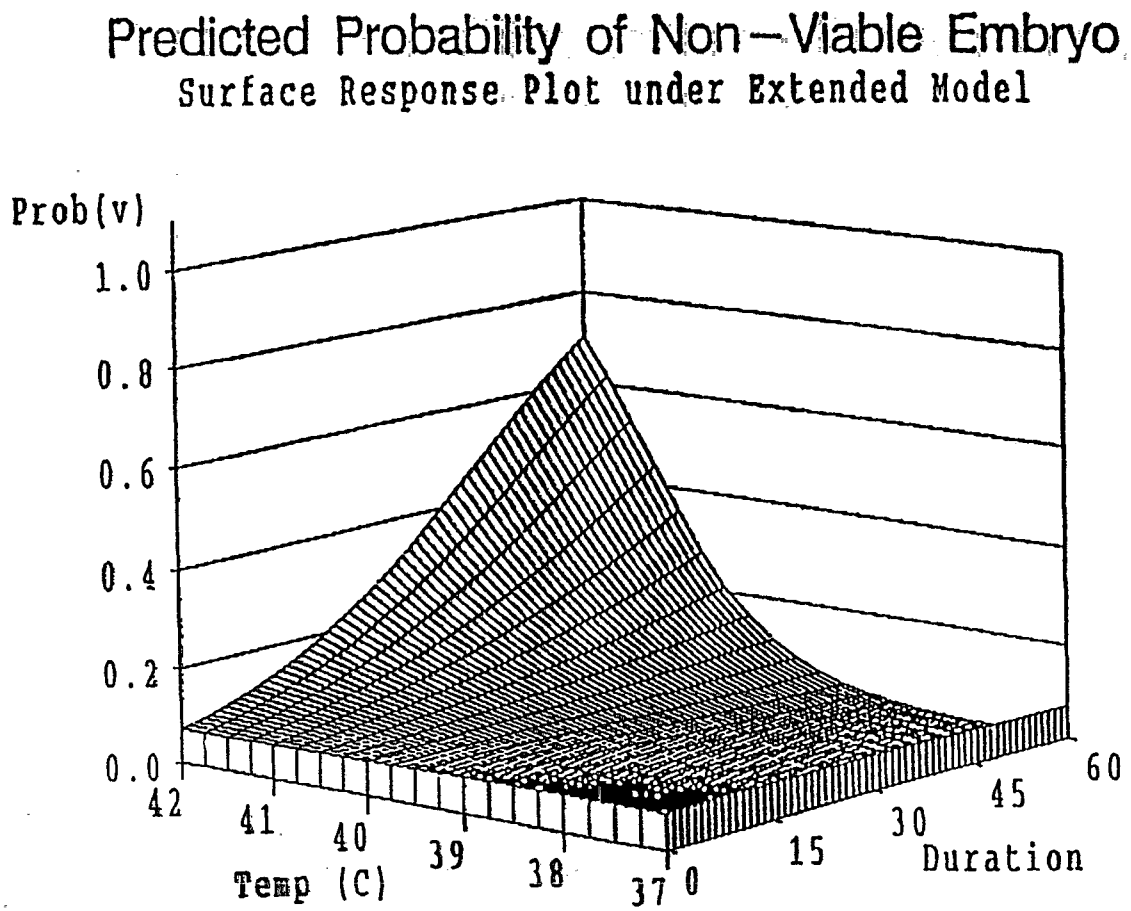

Predicted Probability of Non-Viable Embryo
Surface Response Plot under Extended Model
Prob(v)
1.0
0.8
0.6
0.4
0.2
0.0
42
41
40
39
38
37
Temp (C)
0
15
30
45
60
Duration

Figure 3-3

- To assess whether response depends only on the multiple of "dose" and duration. (None of the responses seen in this study fit Haber's law exactly; all are affected by the extra factors of dose and time.)

Additional long-term objectives include:

- To develop methods for extrapolating to untested exposure intensities or durations
- To apply the models to evaluation of experimental designs

3.2 C × T AND DERMAL TOXICITY

James McDougal, Geo-Centers, Inc., Wright-Patterson Air Force Base

This presentation focuses on the impact of C × T on the dermal route. Two types of dermal toxicity exist: skin as a target organ (local toxicity: irritation, sensitization, corrosion) and skin as a barrier (systemic toxicity).

The skin is the largest organ in the body, made up of the epidermal layers (including the tightly packed stratum corneum, thought to be the barrier) and the dermal layers.

Flux (milligrams per surface area exposed per time) is

$$\text{FLUX} = \frac{DK_m}{\delta} (C_{out} - C_{in})$$

$$P = \frac{DK_m}{\delta}$$

where

D is diffusivity (cm^2/hr)
K_m is membrane partition coefficient (unitless)
δ is membrane thickness (cm)
C is concentration (mg/cm^3)
P is permeability coefficient (cm/hr)

Experimentally, skin is isolated in a diffusion cell (Figure 3-4). The cumulative mass absorbed over time is shown in Figure 3-5, showing that linear absorption occurs after a lag time of 30 to 45 minutes before any of the chemical comes through the skin in to the receptor solution.

How is Haber's law used in the risk assessment? For systemic toxicity, if we look at mass that comes across the skin, the fixed law relationship is:

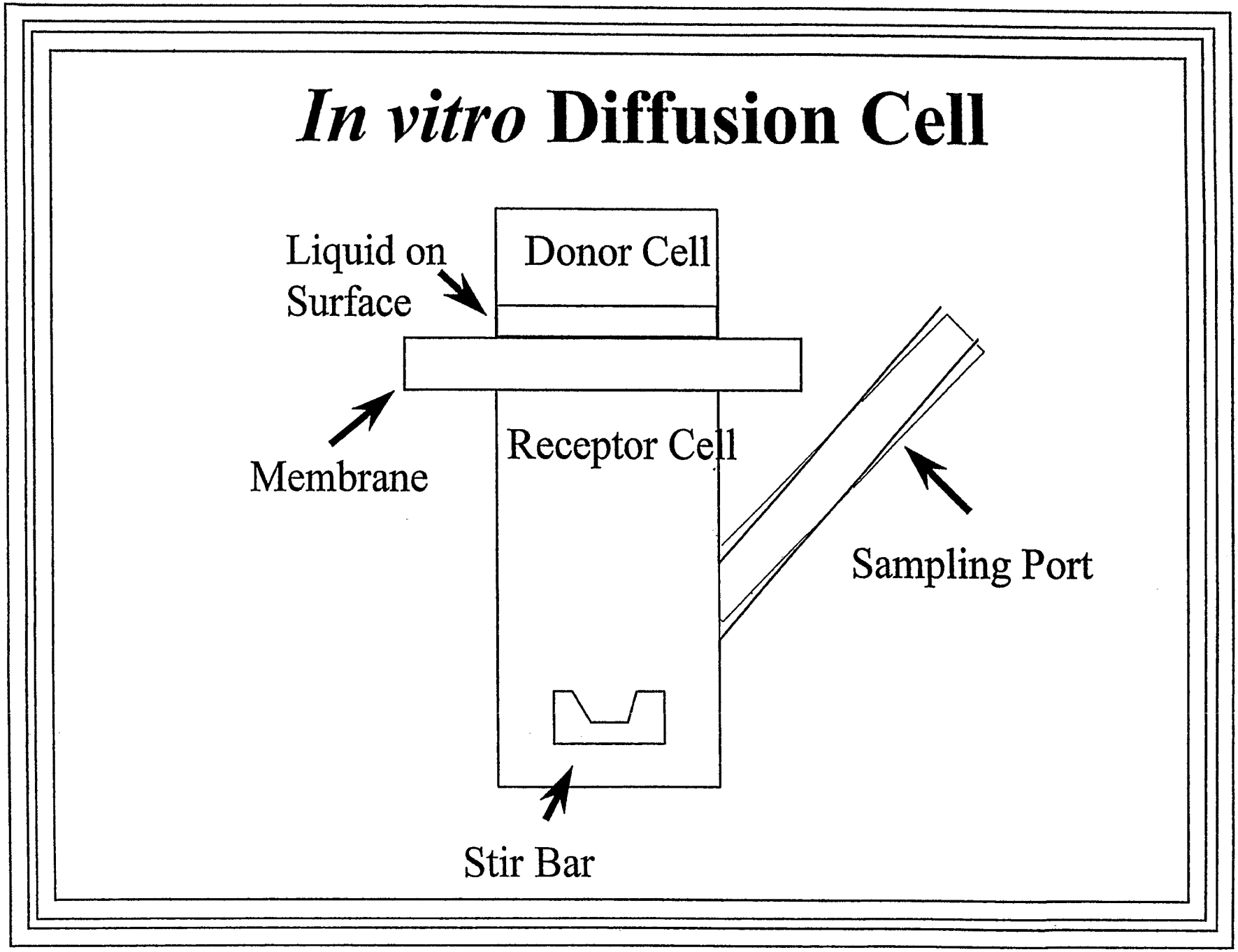

In vitro Diffusion Cell
Liquid on Surface
Donor Cell
Membrane
Receptor Cell
Sampling Port
Stir Bar

Figure 3-4

Lag Time

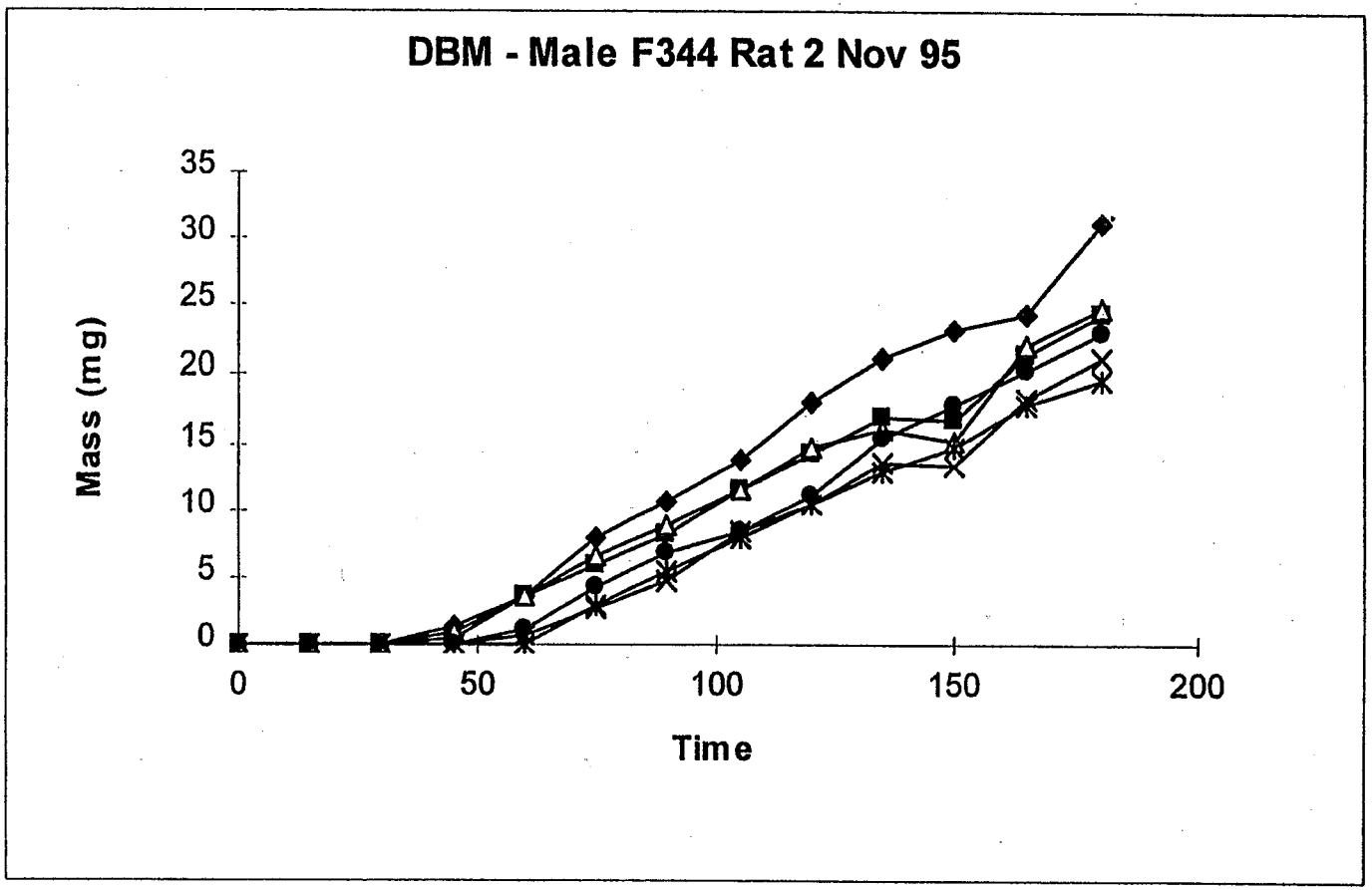


Figure 3-5

$M_{IN} = PACt$

where

M_{IN} is total absorbed (mg)
P is permeability coefficient (cm/hr)
A is area exposed (cm^2)
C is exposure concentration (mg/hr)
t is exposure time (hr)

However, this does not take lag time into account. EPA has recommended the following approach, which includes the chemical in the skin as well as the chemical that comes through the skin:

$$MIN = AC \frac{4(Km\delta)Pt}{\pi} \qquad \text{(For exposure <2.4 lag time)}$$

where Km = membrane (skin) partition coefficient
δ = thickness of skin

For systemic toxicity, C × T ignores lag time and may be very inaccurate (Figure 3-6). For local toxicity, we assume that the toxicity is related to the concentration of chemical in the skin (Figure 3-7). Lag time is not as important; C × T would be inaccurate if the skin is saturated. The way to address toxicity from the dermal route is to use a mechanistic approach, such as PBPK modeling.

With C × T, we always focus on the external concentration, the time of exposure (minutes to years), and some constant toxicity related to the product of the two. But many nonlinear processes occur between external concentration and the response, as shown in Figure 3-8, making C × T inaccurate.

3.3 NEUROTOXIC EFFECTS OF TRICHLOROETHYLENE INHALATION AS A FUNCTION OF EXPOSURE CONCENTRATION, DURATION, AND TARGET TISSUE DOSE

William Boyes, EPA National Health and Environmental Effects Research Laboratory

This project examined concentration-duration relationships for inhalation exposure to trichloroethylene (TCE) as a model volatile organic solvent, and examined the tissue dosimetry as a better predictor of the outcome.

The study looked at three different outcome measures of neurotoxicity: hearing loss (a permanent effect, due to damage to the cochlea, following exposure to high concentrations for an extended duration) as well as two acute, reversible effects: visual function and signal detection behavior. Tissue dose was estimated using a classically based PBPK model.

Figure 3-9 shows some of the results. These data show that Haber's rule is underprotective when going from long- to short-duration exposures and overprotective when going from short- to long-duration exposures.

Systemic Toxicity will be Related to Internal Concentration

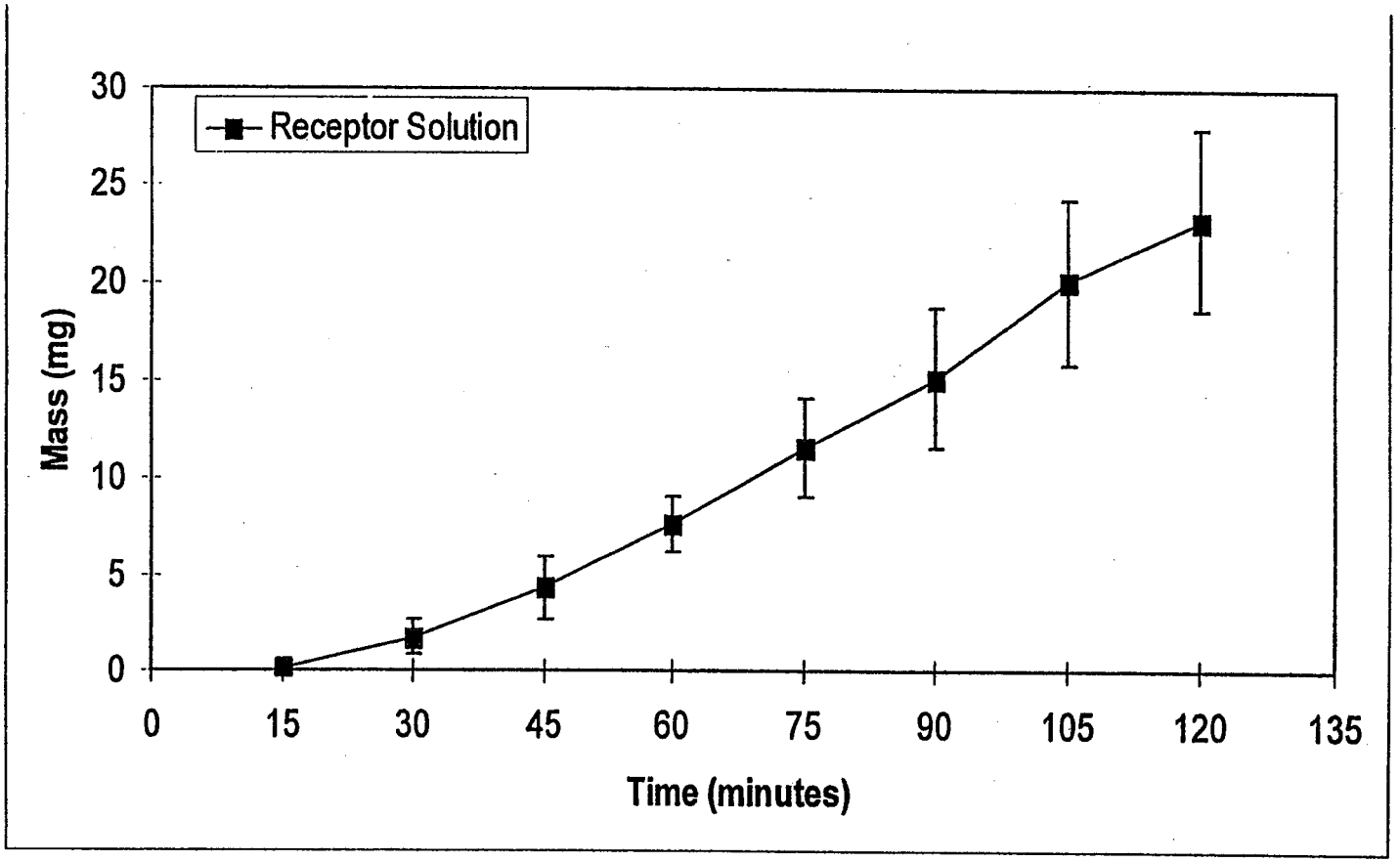


Figure 3-6

Local Toxicity will be Related to Tissue Concentration

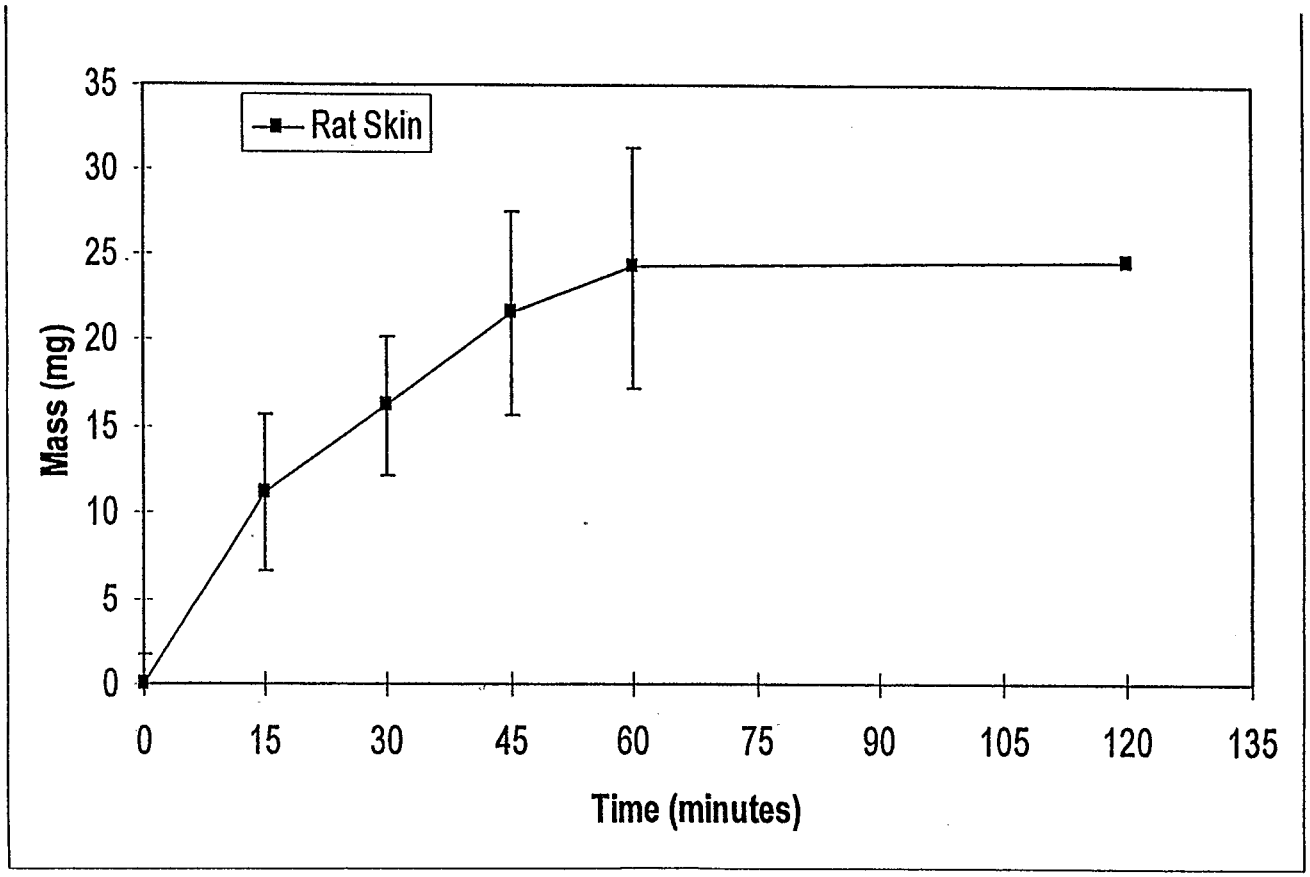


Figure 3-7

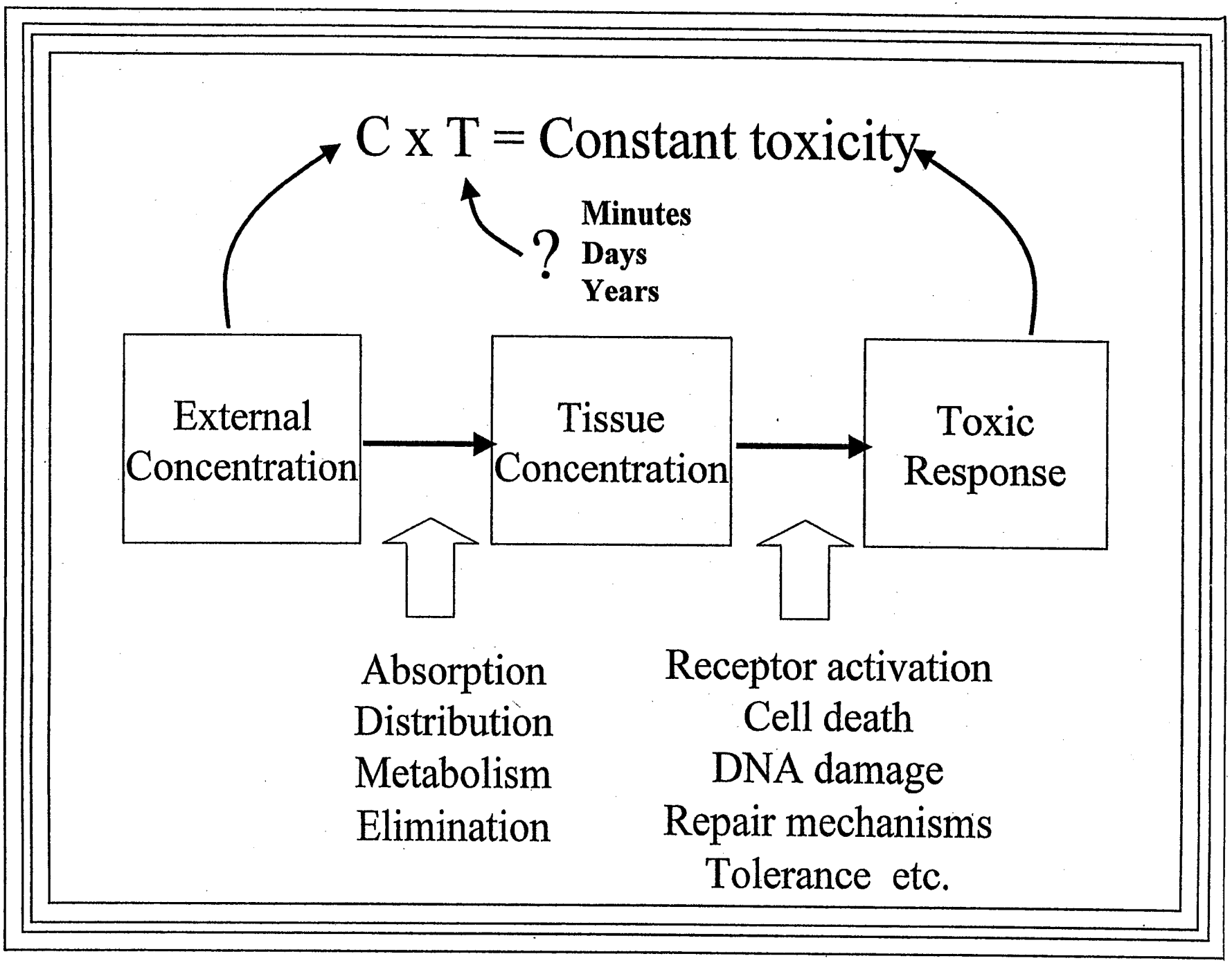

C x T = Constant toxicity
?
Minutes
Days
Years
External Concentration
Tissue Concentration
Toxic Response
Absorption
Distribution
Metabolism
Elimination
Receptor activation
Cell death
DNA damage
Repair mechanisms
Tolerance etc.

Figure 3-8

Haber's Predictions vs Observed Outcomes

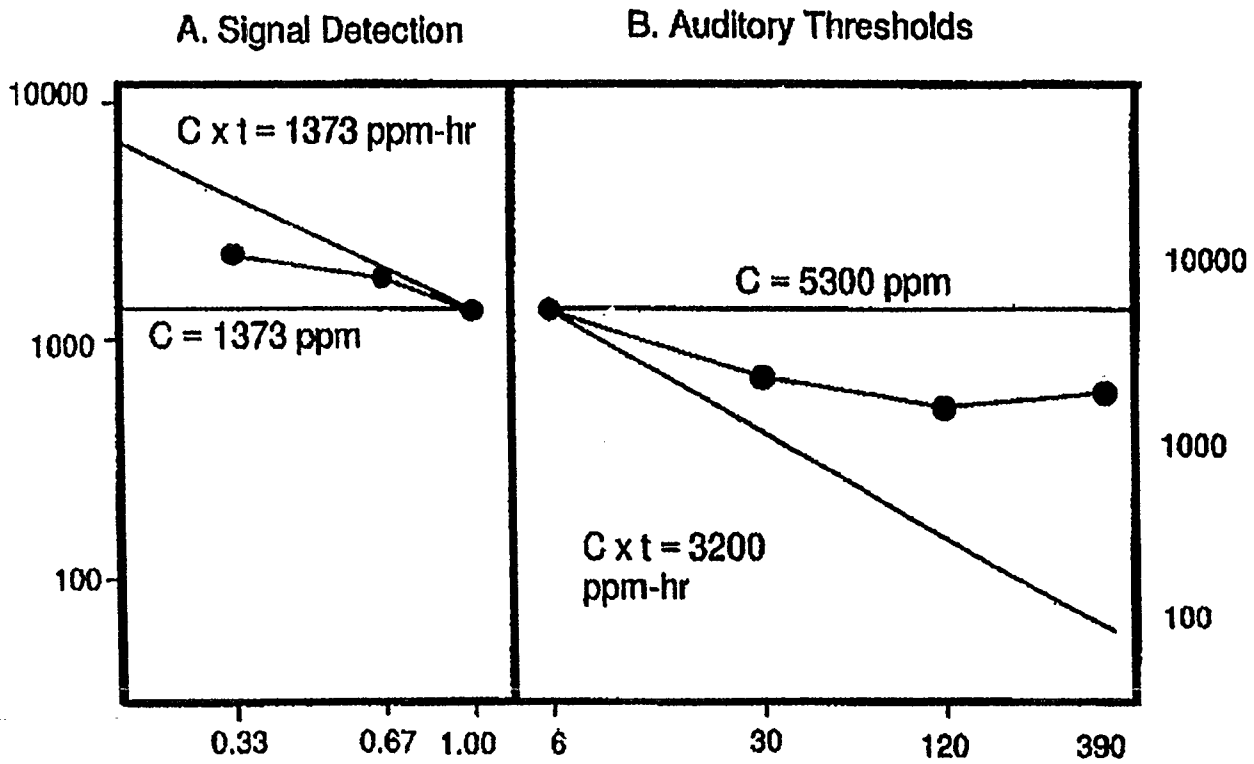


Figure 3-9

Tissue dosimetry might allow better predictions. Figure 3-10 shows the four exposure situations in the experimental design, which allows evaluation of what the proper dose metric is for evaluating outcomes (area under the curve, peak concentration, or C × T).

Visual effects showed poor relationship to area under the curve (AUC arterial [TCE]) but a good relationship to peak concentration (Figure 3-11). Figure 3-12 shows that it doesn't matter what concentration or duration is used; the magnitude of effect in the brain can be predicted as a function of the blood concentration. This shows that the momentary or peak concentration of the compound is the critical determinant of the effect.

Figure 3-13 shows predictions of PBPK model; concentrations in blood versus brain (target tissue); and brain concentration versus outcome. One can go from any combination of atmospheric data through a series of mathematical relationships to predict quantitatively the magnitude of effect in the brain. Figure 3-14 is a simplified version that could be used for risk assessment to see when effects on brain function are likely to occur. This figure is based on values determined in rats, and would have to be converted into equivalent human values before being used for risk assessment.

The study concluded that:

- The linear form of Haber's rule led to inaccurate extrapolations across durations.
- The tissue dose estimated from the PBPK model predicted well across exposure conditions.
- Peak tissue concentration was an appropriate measure for acute TCE effects.
- Other mechanisms of action may require other tissue-dose metrics.

3.4 RESPIRATORY TOXICITY: COHERENT RESPONSE MODELS OF OZONE INJURY IN HUMANS AND ANIMALS

Dan Costa, EPA National Health and Environmental Effects Research Laboratory

Haber's law as we use it is probably somewhat of a misrepresentation; Haber used it specifically for lethality resulting from pulmonary effects, and he said it was only useful when comparing two toxicants but not when looking within a toxicant for C × T relationships.

For ozone, percent mortality versus concentration of ozone shows a reasonable C × T relationship. Ozone reacts with lung tissue immediately. In inhalation toxicology studies, we are limited in design, and are generally dealing with a square wave type of exposure. In environments such as Los Angeles, ozone concentrations go up and down along with other toxicants. A year ago the ozone standard was reconsidered. It was historically established using a 1-hour peak concentration, but concern exists about possible cumulative effects from an 8-hour exposure.

This study looked at a direct measure of toxicity: protein leakage from the vascular side to the pulmonary side. In looking at a number of species, modeling the pulmonary tissue dose using the Miller-Overton model, a linear response exists. A C × T matrix exposure was done with animals. For the rat and guinea pig, an exponential model fit the data pretty well:

Estimated Blood [TCE]

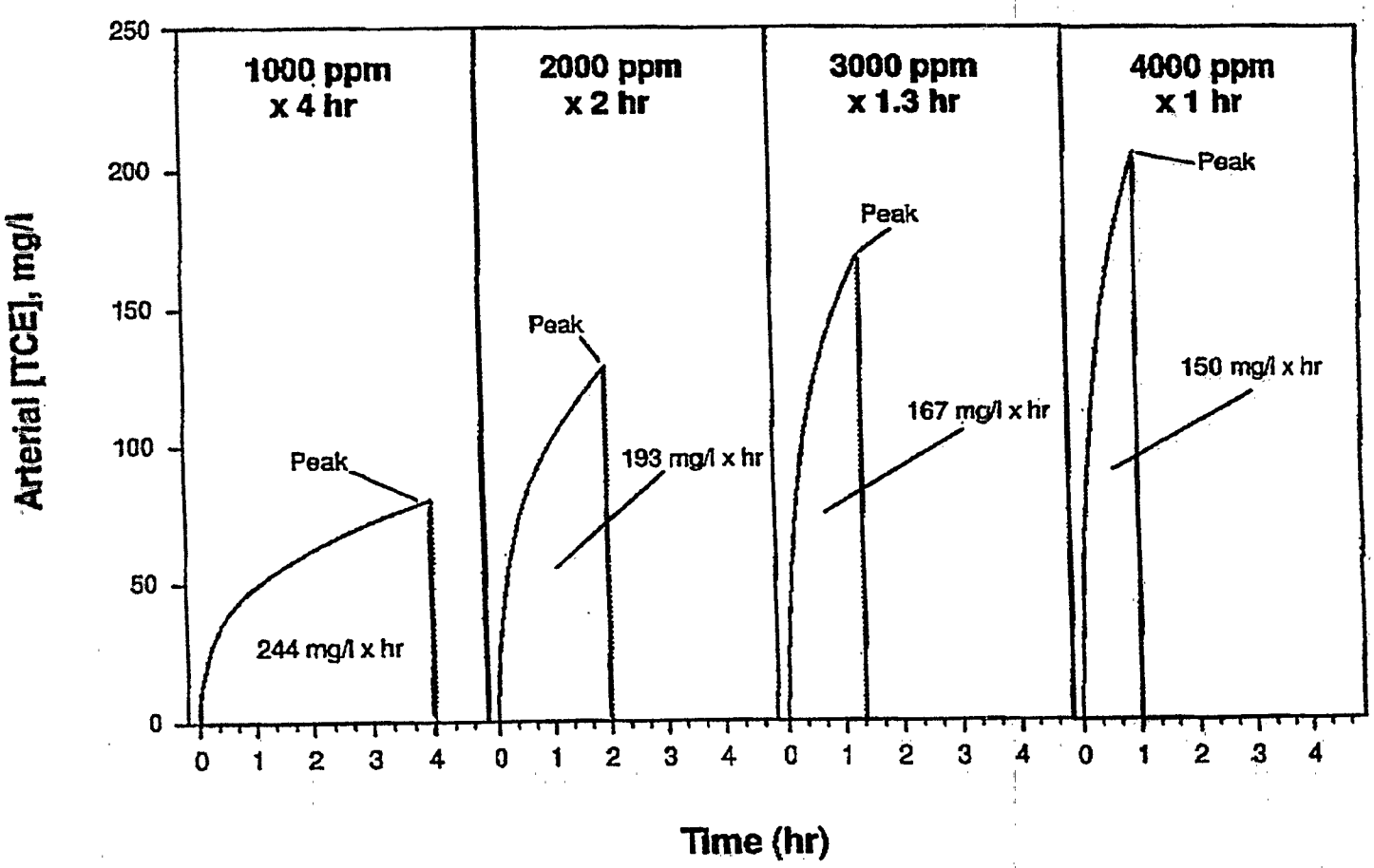


Figure 3-10

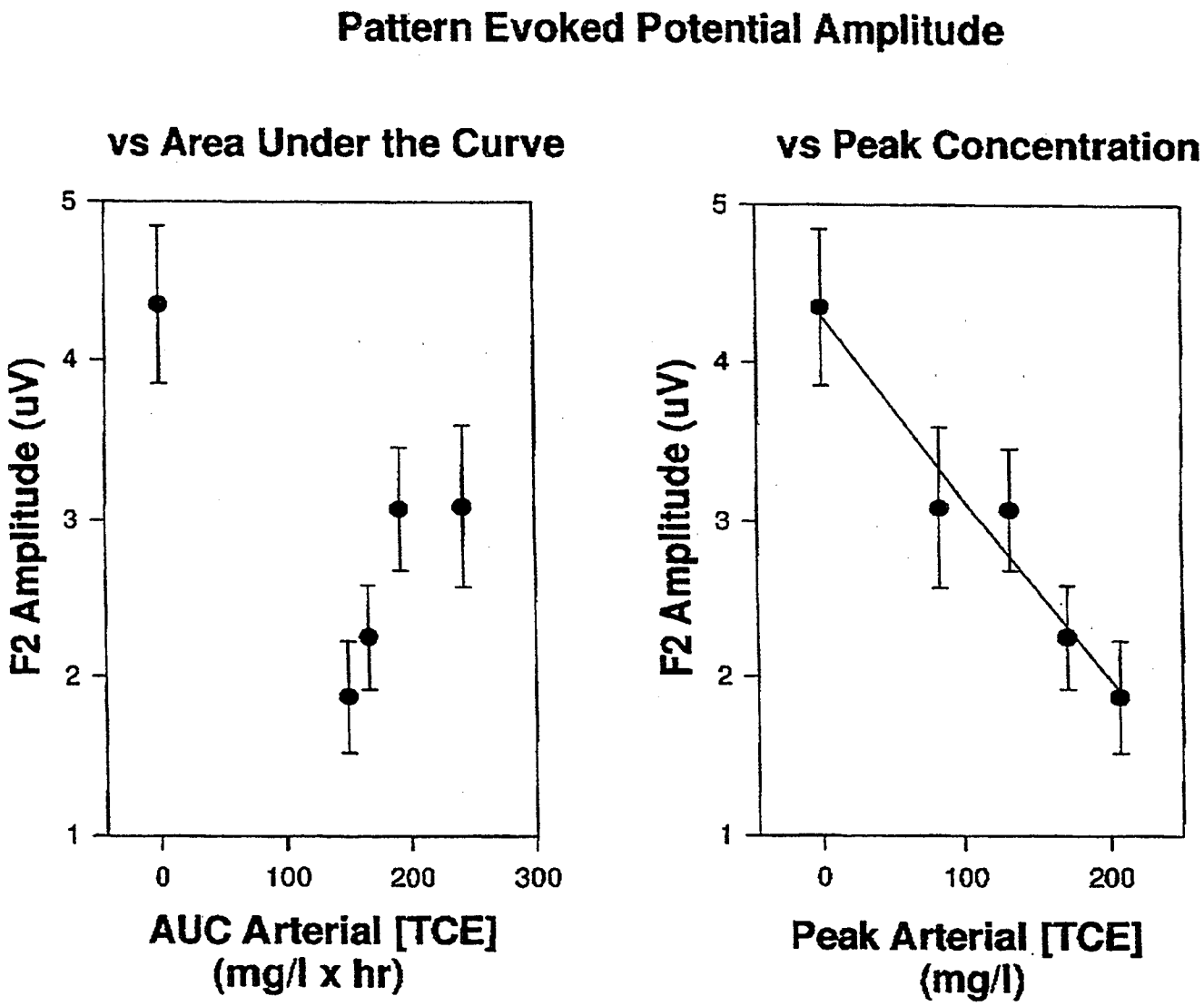

Pattern Evoked Potential Amplitude
vs Area Under the Curve
vs Peak Concentration
F2 Amplitude (uV)
5
4
3
2
1
0
100
200
300
AUC Arterial [TCE]
(mg/l x hr)
Peak Arterial [TCE]
(mg/l)

Figure 3-11

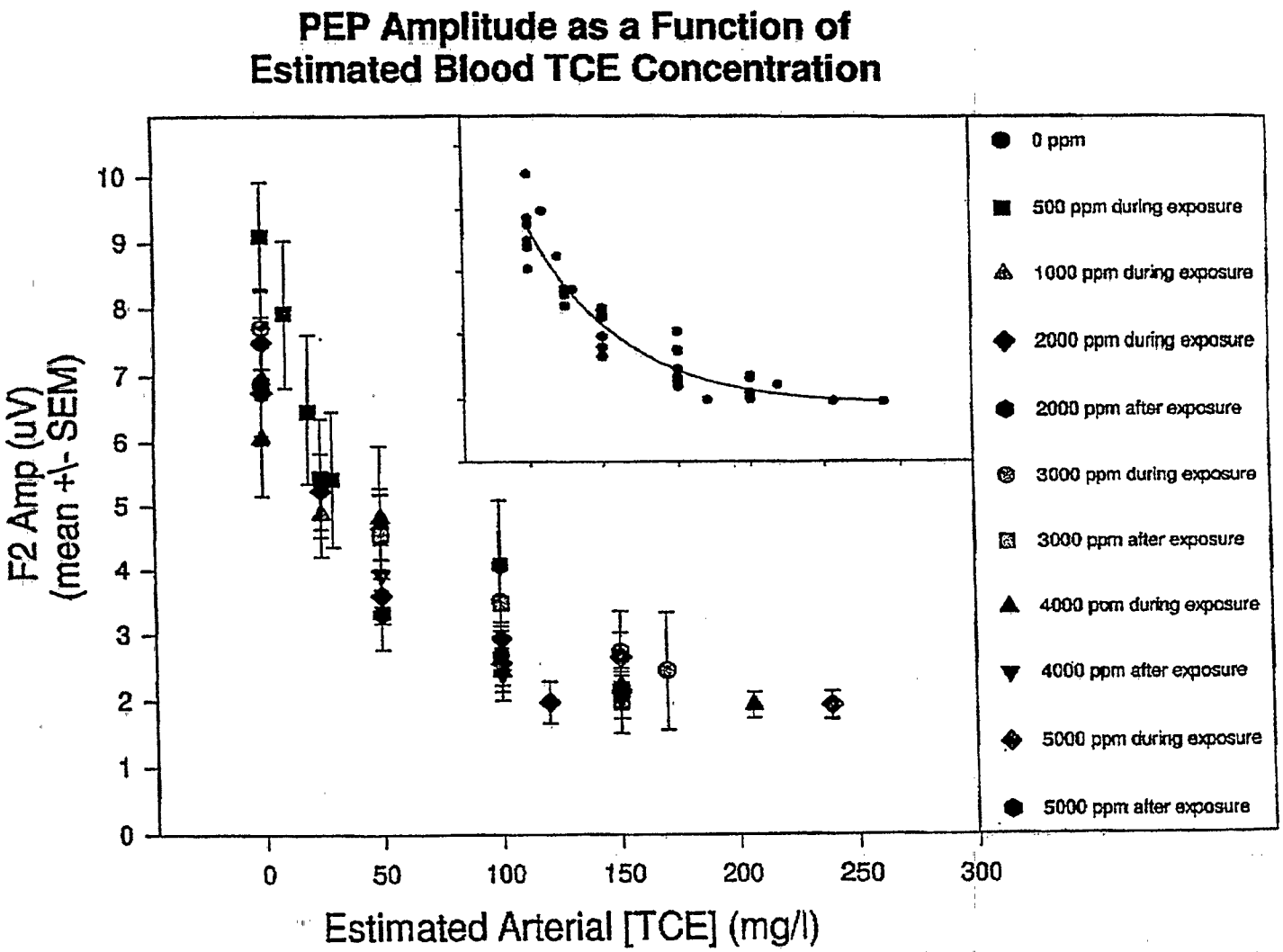

PEP Amplitude as a Function of
Estimated Blood TCE Concentration
F2 Amp (uV)
(mean +\- SEM)
10
9
8
7
6
5
4
3
2
1
0
0
50
100
150
200
250
300
Estimated Arterial [TCE] (mg/l)
0 ppm
500 ppm during exposure
1000 ppm during exposure
2000 ppm during exposure
2000 ppm after exposure
3000 ppm during exposure
3000 ppm after exposure
4000 ppm during exposure
4000 ppm after exposure
5000 ppm during exposure
5000 ppm after exposure

Figure 3-12

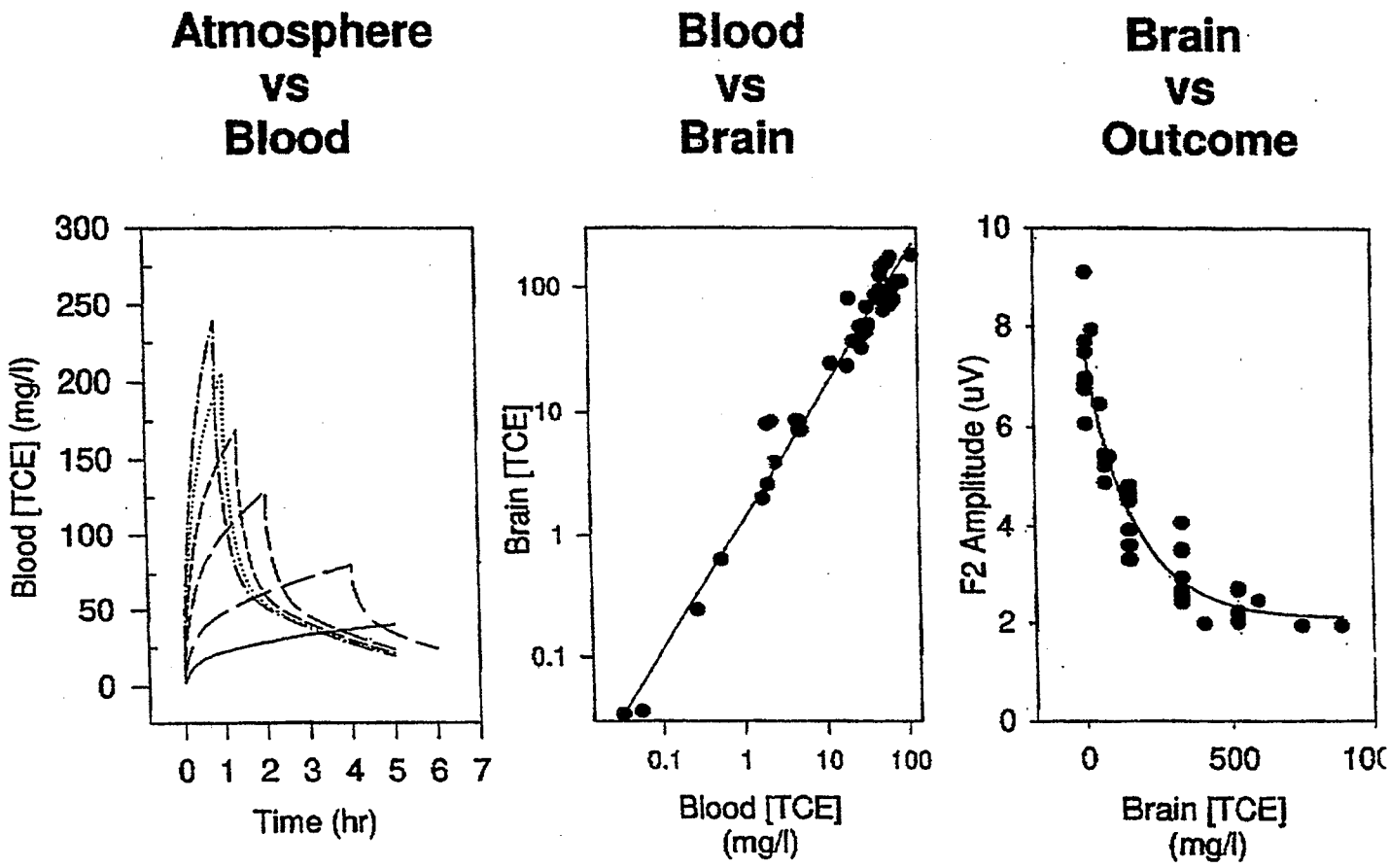

Atmosphere
vs
Blood
Blood
vs
Brain
Brain
vs
Outcome
Blood [TCE] (mg/l)
300
250
200
150
100
50
0
0 1 2 3 4 5 6 7
Time (hr)
Brain [TCE]
100
10
1
0.1
0.1 1 10 100
Blood [TCE]
(mg/l)
F2 Amplitude (uV)
10
8
6
4
2
0
0 500 100
Brain [TCE]
(mg/l)

Figure 3-13

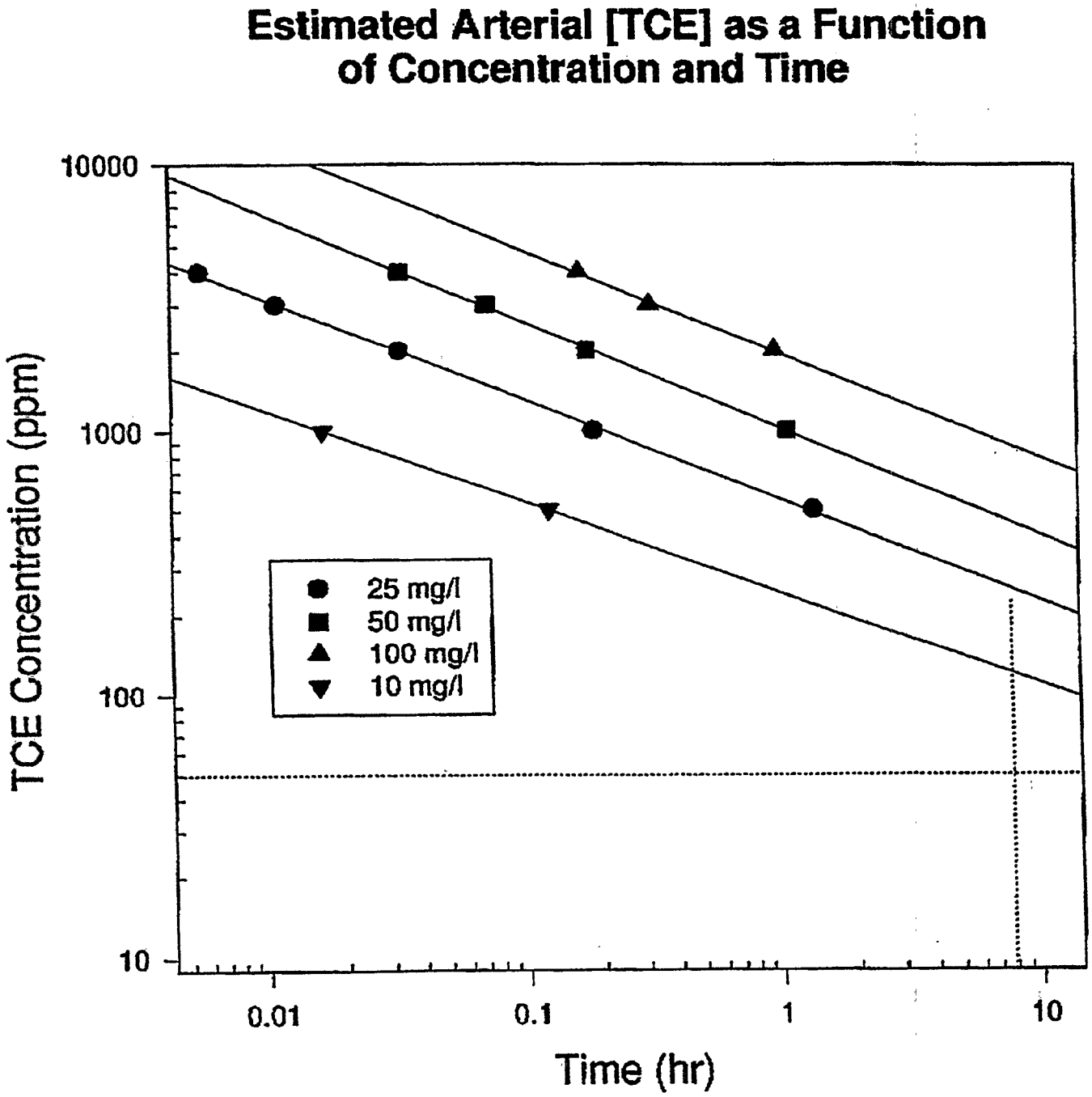

Estimated Arterial [TCE] as a Function of Concentration and Time
10000
1000
100
10
TCE Concentration (ppm)
0.01
0.1
1
10
Time (hr)
25 mg/l
50 mg/l
100 mg/l
10 mg/l

Figure 3-14

F-344 Rat

$$\ln BALP = 4.59 + 0.47e^{0.147CT}$$

Guinea pig

$$\ln BALP = 4.59 + 0.94e^{0.147CT}$$

$$R^2 = 0.86$$

This was applied to other endpoints where human as well as animal data were available. A major database exists on reduction of forced vital capacity, from which the 1-hour ambient standard for ozone was established. The FVC falls with increasing concentrations of ozone (Figure 3-15). Three primary human models describe this data set (Figure 3-16).

The exponential model (Response = $Ae^{C \times T}$) is preferable because it is simple, yet fits the data well. In addition, it:

- Describes first-order chemical processes conceptually similar to O_3 substrate reaction
- Describes many concentration-related events (e.g., uptake, elimination in PK models)
- Describes population responses (e.g., growth)
- Utilizes C × T as simple exposure parameters

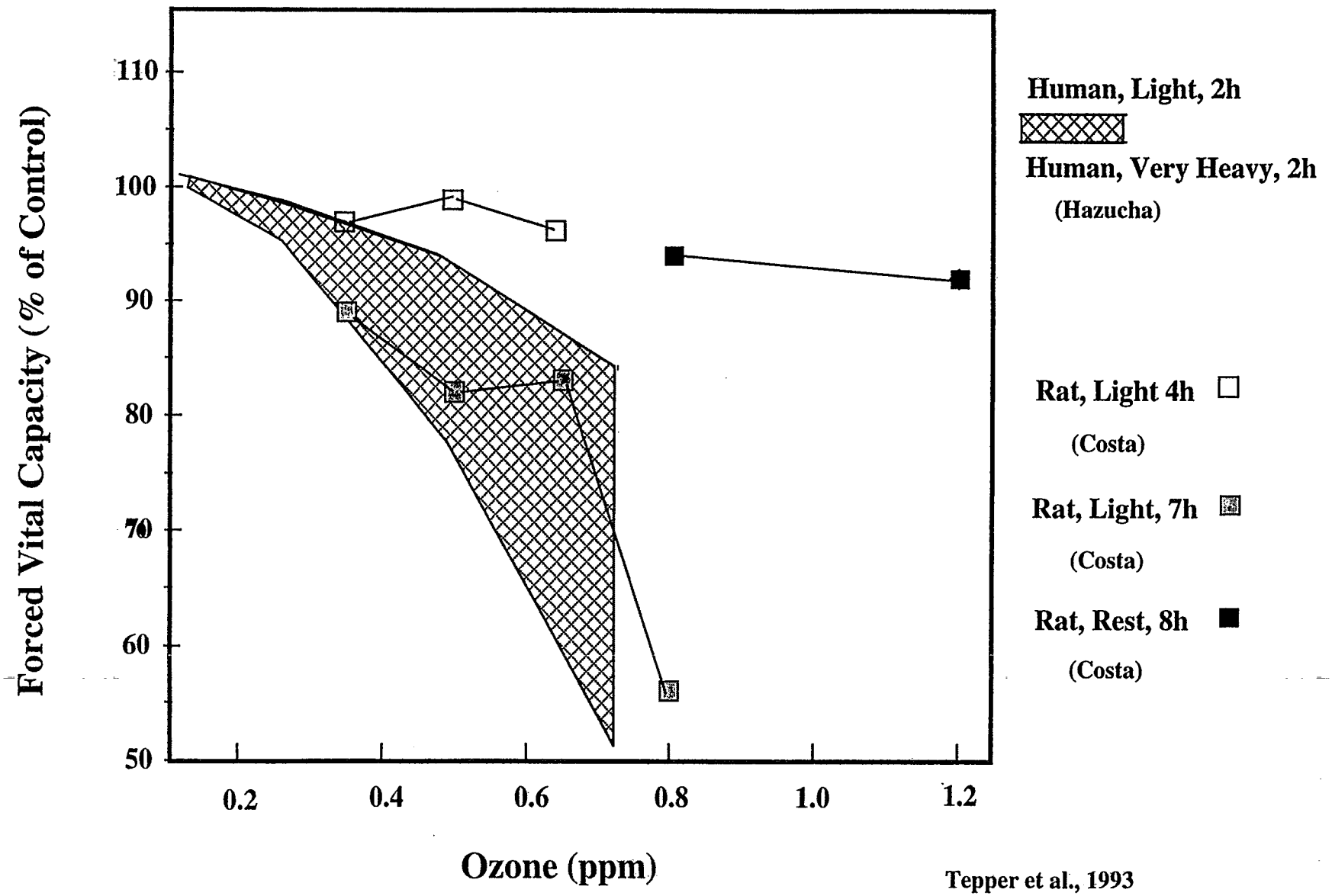
The application of the exponential model to animal and human data is illustrated by the relationships expressed in Figure 3-17 and shown graphically with human data from previously published studies in Figure 3-18. The one place where the exponential model begins to fail is at high concentrations and high levels of exercise where one might assume high doses. In chronic studies looking at thickening of distal airways, a simple C × T relationship works pretty well when a dosimetry model is incorporated that accounts for losses of ozone down the airways.

The study concluded that in the case of ozone:

- C × T appears to be a reasonable approximation for dose, especially at lower concentrations.
- For a single-day exposure, the exponential relationship of response for C × T appears to describe adequately across species.
- The adequacy of the C × T dose metric may be endpoint dependent.
- Where the endpoint can be mechanistically linked to a cumulative injury/stimulus, C × T may be appropriate for chronic outcomes.

For a particularly corrosive inhalant such as phosgene (or one with diverse mechanisms/responses), concentration may overshadow the impact of exposure duration on the magnitude of response.

O_3 -Induced FVC Changes in Humans & Rats



Tepper et al., 1993

Figure 3-15

Three Primary Human Models

(1) Logistic Models (McDonnell et al., 1983, '93, '97)

$$DELFEV_1 = \frac{B_1(1-B_2 Age)}{1+B_5 e^{-B_3(CV_E^{B_4})(1-e^{-B6T})}}$$ (includes age & exercise)

(2) Quadratic model (Hazucha, 1987)

Before / After = 1- $0.378C^2$ (light exercise)

Before / After = 1- $1.170C^2$ (heavy exercise)

(3) Log regression (Larson et al., 1991)

1 - Before / After transformation to z score

$z = -0.175 + 0.911 \ln T + 1.202 \ln C$

Figure 3-16

Application of an Exponential Model on Selected O_3 Data-Sets

F. 344 Rat	
Highfill (91)	$PROT = 149e^{0.136C \times T}$
Tepper (91) (w/CO_2)	$PROT = 1604e^{0.134C \times T}$
	$FVC = 9.69e^{-0.069C \times T}$
Wistar Rat	
Rombout (89)	$PROT = 90.8e^{0.144C \times T}$
	$(C < 0.75 \text{ ppm})$
Guinea Pig	
Highfill (91)	$PROT = 249e^{0.198C \times T}$
	(w/o 0.8 ppm) $250e^{0.137C \times T}$
Human	
Devlin (91)	$PROT = 192e^{0.164C \times T}$
Horstman (90)	$FEV_1 = 4402e^{-0.136C \times T}$
Folinsbee (88)	$FEV_1 = 4245e^{-0.180C \times T}$
	$FEV_1 = (\Sigma B_n S_n)e^{-0.61(S10C \times T - 0.132(S2-S9)C \times T}$

Figure 3-17

Model versus Data for the Human

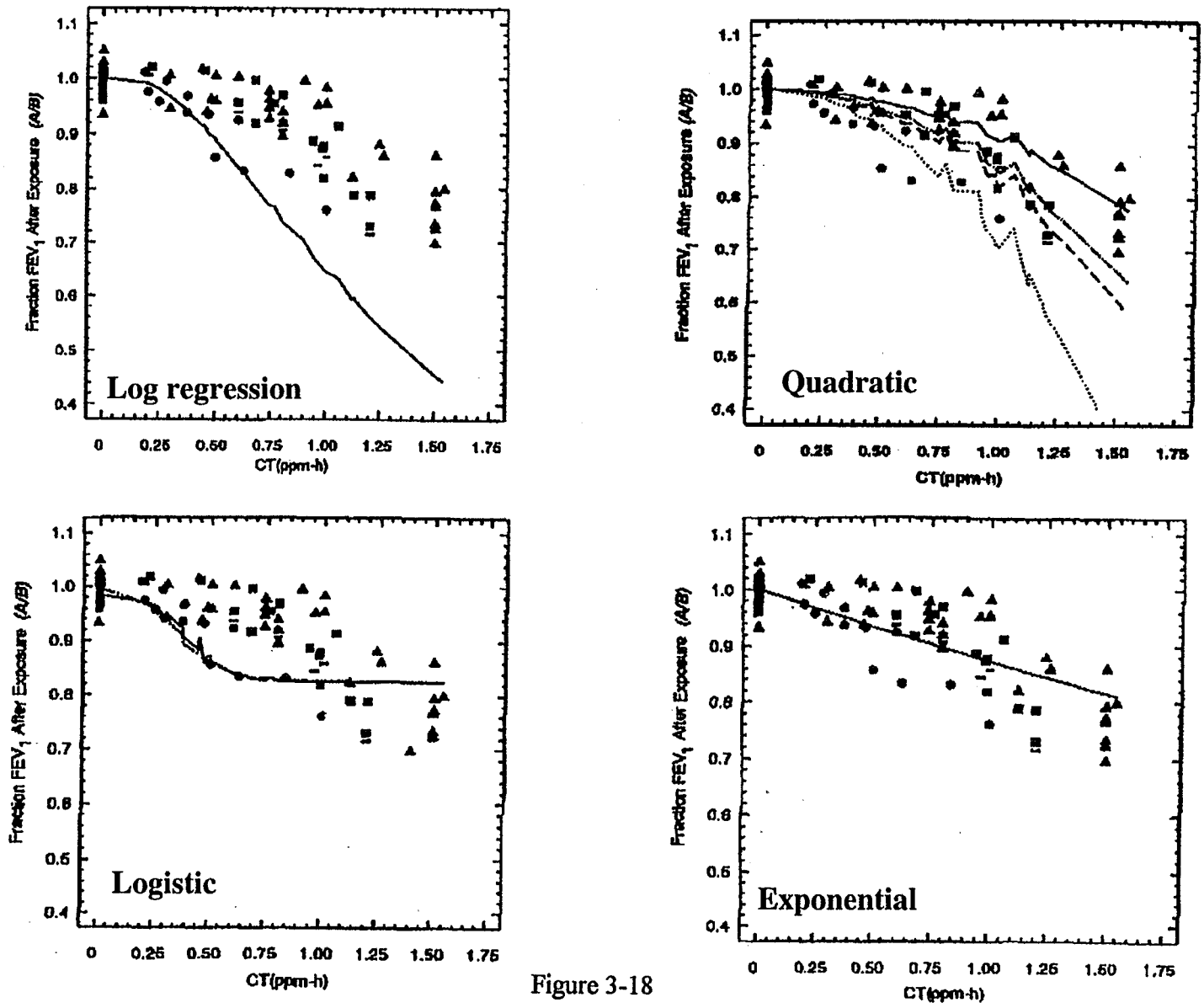


Figure 3-18

3.5 OBSERVER COMMENTS

- Dr. Chen from OSHA discussed the importance of having a description of rate (what are the circumstances and assumptions?). Clearly communicating the end result of risk assessment to the public is critical. With regard to gaining more transparency in the risk assessment, it is has been stated that it is important to conduct more research on metabolism and mechanism. Good examples are butadiene and methylene chloride, for which this research has been done in the last few years but the risk assessment is not better.

- Dr. Strickland from NCEA presented categorical regression as a way to look at $C \times T$. The software/documentation for the CatReg model is available from EPA (EPA/600/R-98/052 and EPA/600/R-98/053).

- Ernest Falke noted that when doing $C^n \times T = K$, one should consider the impact on risk assessment. The higher the value of n, the flatter the dose-response curve. If $n = 1$, an interpretation could be that there is no repair. Finally, some acute studies present data in terms of CT products; this is transformed data that is difficult to look at.

- Carol Kimmel raised a question about the models presented in which the effect of duration at low doses is much less than at higher doses. Since we are using the lowest dose or NOEL for extrapolation to lower levels; correction on a $C \times T$ basis will result in overcorrection. This should be taken into account in default situations.

SECTION FOUR

PLENARY PRESENTATIONS: STATISTICAL APPROACHES

4.1 WHAT CAN MECHANISMS TELL US ABOUT MODELING DOSE TIME RESPONSE RELATIONSHIPS?

Dale Hattis, Clark University

Mechanistic models are important and potentially helpful because:

- They are potentially true (capture some real, albeit simplified aspect of the toxic system), allowing projection to unmeasured circumstances of dose, time, etc.
- They are productive of experimental studies/hypotheses in the form of measurable intermediate parameters between external dose and effect.
- When they fail, the way they fail can yield useful mechanistic insights for revising the theory, and for further experimental study.

Using the example of chlorine, the story of the Michaelis-Menten enzyme equation illustrates mechanistic reasoning. The basic Michaelis-Menten framework for saturation of either activation or detoxification processes is:

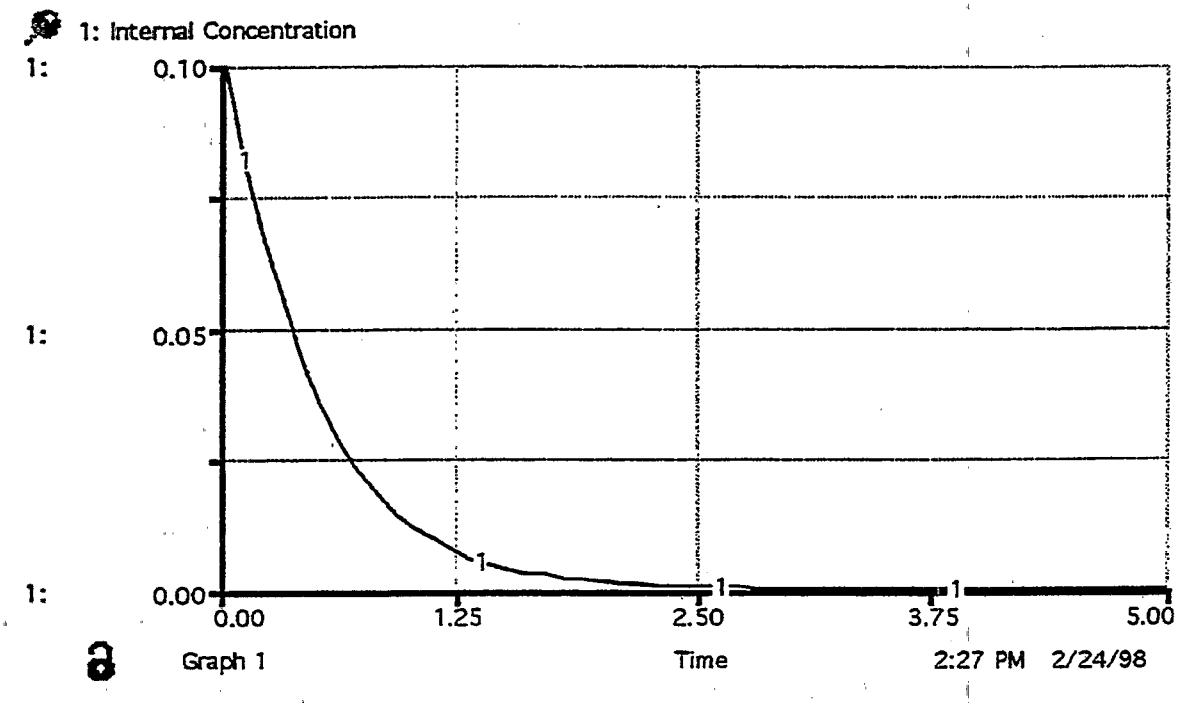
$$\text{Reaction Rate} = \frac{V_{max}\,[C]}{K_m + [C]}$$

This is not an empirical equation, but is derived from fundamental mechanical principles. This is a reaction catalyzed by a few large molecules. At the limit of low doses, the reaction rate is governed by rare chance meetings between the substrate and the active site of the enzyme, so at low doses C in the denominator becomes low relative to K_m and the reaction becomes first order. At high doses, C becomes high relative to K_m and the reaction proceeds at a maximum rate. This can influence dose-time relationships in different ways, depending on whether the damage or the effect depends on C at some relevant receptor site or the integral of C × T (area under the curve).

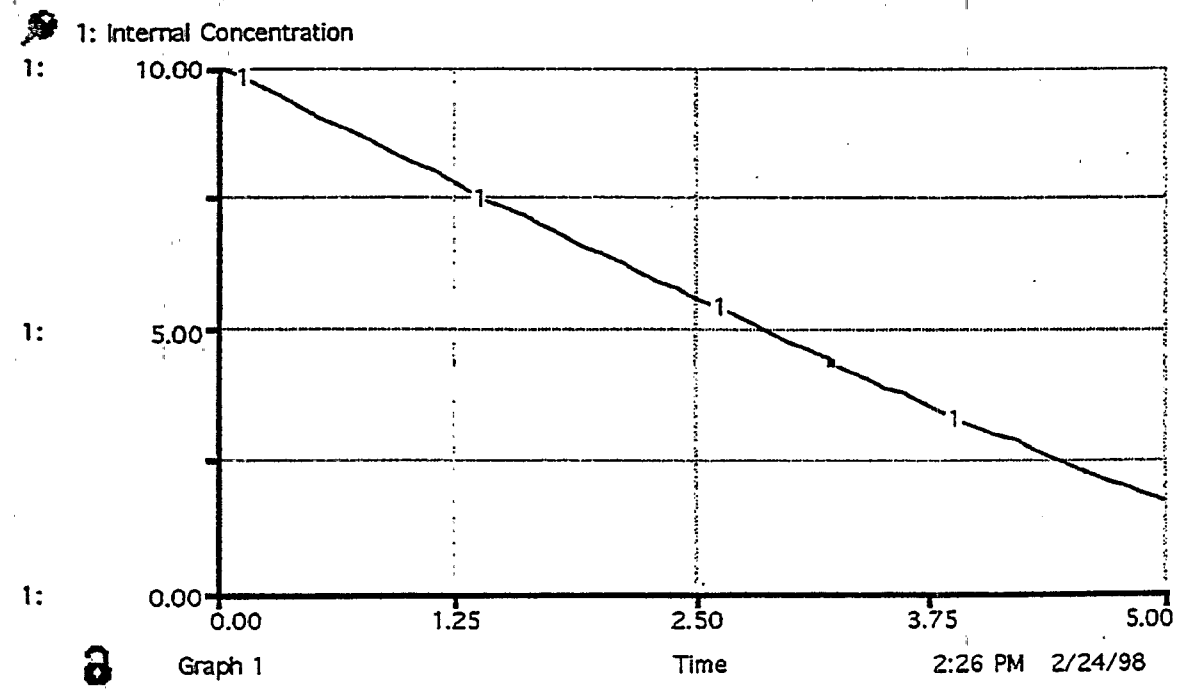
How would different levels of administration affect the C × T product where you have a Michaelis-Menten detoxification process? Figure 4-1 shows Michaelis-Menten metabolism starting either well below K_m (Haber's law applies, repair is first order) or well above (concentration declines at rate of V_{max}, linear over time, leading to a C^2 dependence for the area under the curve).

If linear repair of damage occurs, Haber's law fails at lower doses at the high time end. The rate of effect reversal is critical in understanding these dynamics. Figure 4-2 shows the effects of pulses of exposure building up to C_{max}. Spacing the pulses far apart and compressing the dosing increases C_{max} significantly. Spacing the pulses closer together relative to the biological half-life of the damage or the toxicant does not have the same effect, and C_{max} is proportional to C × T. (Figure 4-3). Therefore, knowing something about

Michaelis-Menten Metabolism Starting Either Well Above or Well Below the Michaelis Constant



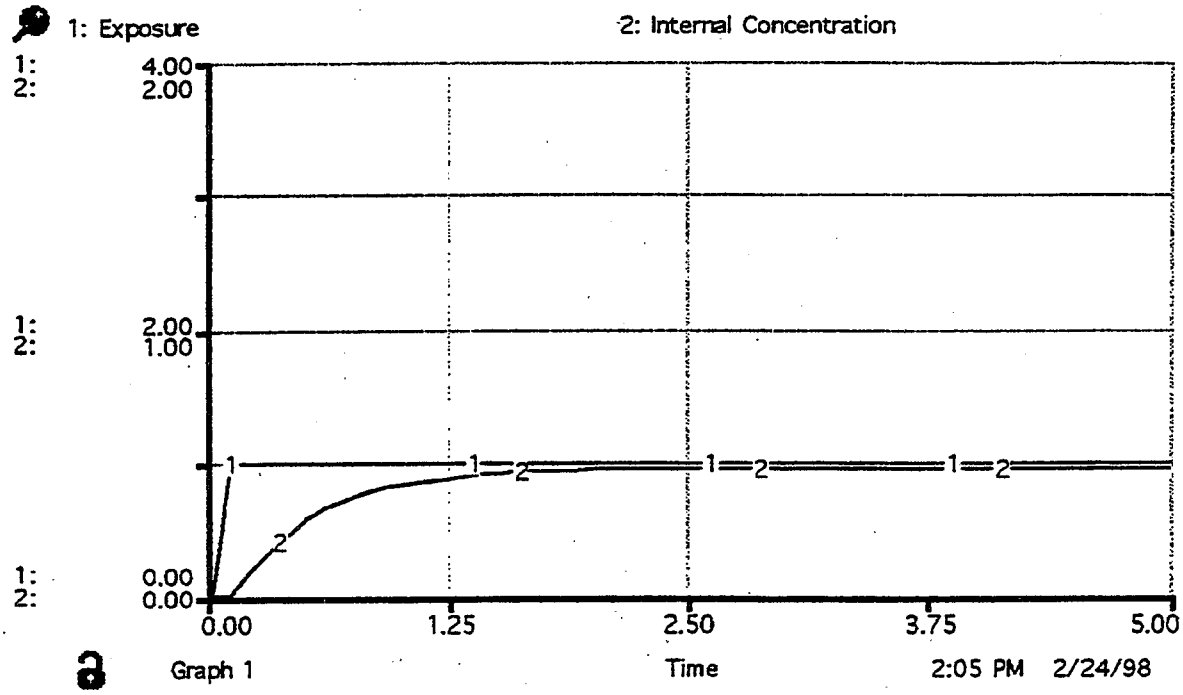
Initial Concentration = 0.1 X Michaelis Constant



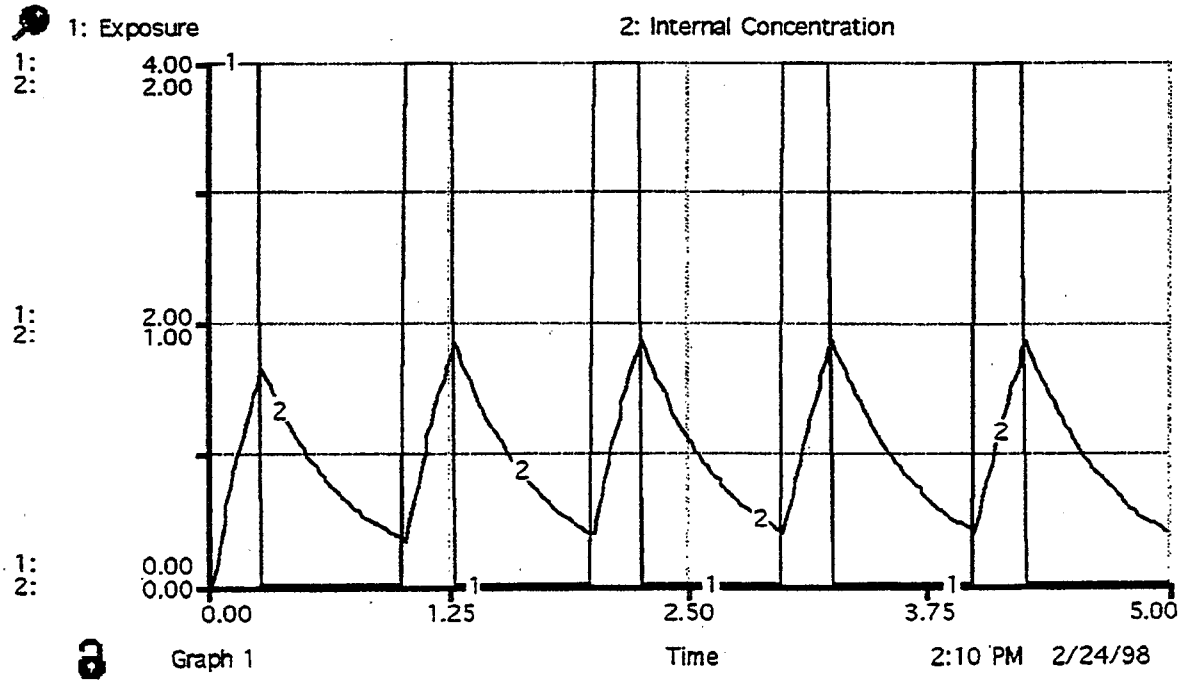
Initial Concentration = 10 X Michaelis Constant

Figure 4-1

Effects of Pulses Spaced At Intervals of 3 Half-Lives



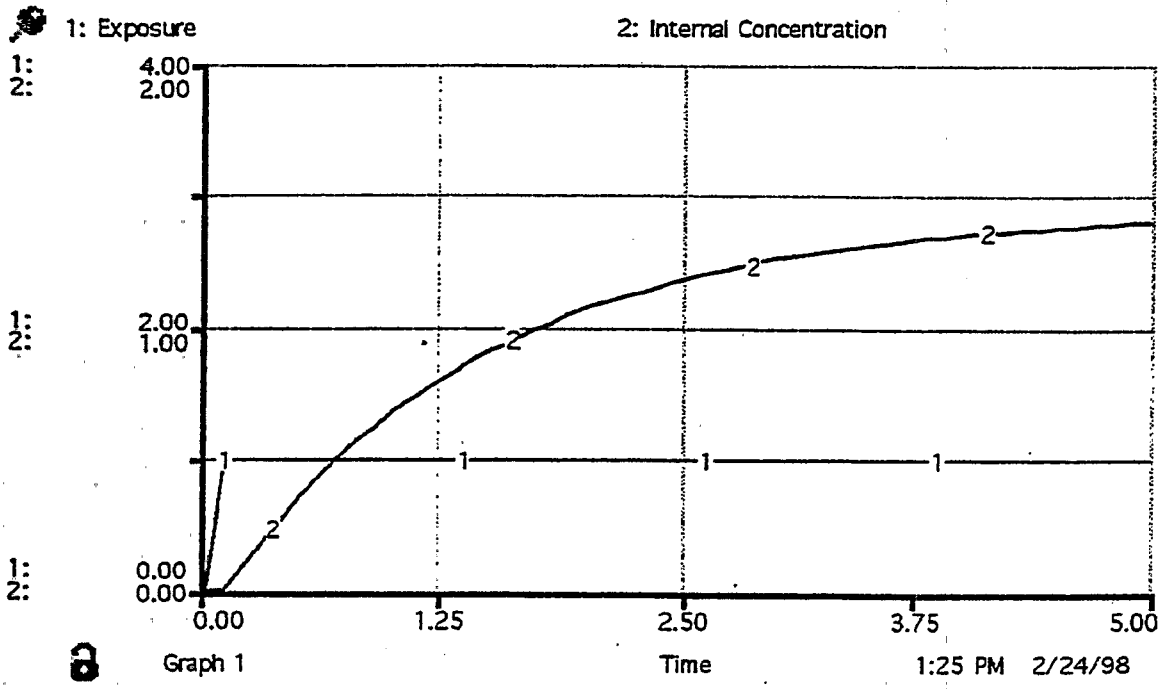
Constant exposure, Half-Life = 1/3, Cmax = .48



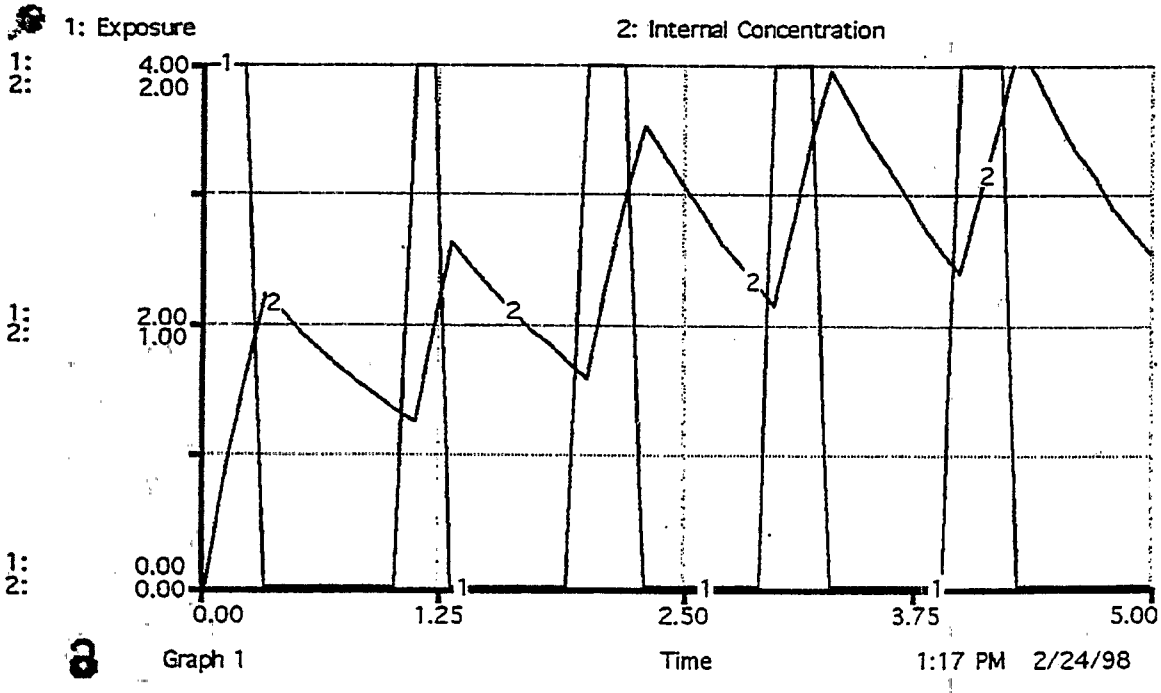
4X exposure rate for 1/4 time; Cmax = .94

Figure 4-2

Effects of Pulses Spaced At Intervals of 1 Half-Life



Constant exposure, Half-Life = 1, Cmax = 1.4



4X exposure rate for 1/4 time; Cmax = 2.1

Figure 4-3

the dynamics of reversal for reversible processes help in predicting when Haber's law will apply and how much adjustment is needed.

It is important to know the biological mechanisms, and they need to work at multiple levels of biological organization, depending on where the important dynamic processes are occurring. This is not a statistical problem, but a problem of understanding biology quantitatively. At the biochemical/molecular level, examples include:

- Enzyme inhibition or unproductive reactant yielding too much or too little of some vital function. This can be irreversible (e.g., phosphate anticholinesterase) or reversible (e.g., uncoupling of oxidative phosphorylation by arsenic).
- Receptor agonism, i.e., too much of a signal at a key developmental point.
- Receptor antagonism, i.e., too little of a signal at a key developmental point.

At the organelle or cellular level, examples include:

- Depletion of a key cofactor (e.g., glutathione) or energy resource (ATP) leading to temporary loss of function (such as neurotransmission) or cell death
- Death of nonreplicating cells (e.g., neurons) as opposed to loss of cells with a normal replacement cycle
- Disturbance of appropriate differentiation pathways
- Inappropriate triggering of apopstosis (or inappropriate inhibition of programmed cell death, which may be involved in producing teratogenic effects)

At the tissue/organ level, long-term adaptive responses can occur, including:

- Proliferation of goblet cells leading to chronic bronchitis
- Irreversible loss of neurons in the substantia nigra, leading to inadequate internal concentrations of dopamine and loss of control function
- Irreversible loss of lung support structural proteins and alveolar septa, leading to emphysema/decreased lung function

At the systemic level, problems include:

- Inadequate oxygen transport by carbon monoxide inhibition of oxygen binding to red cells, leading to impaired function and death of the brain and other organs
- Dynamic consequences of enzyme induction, including enzymes that induce activation or inactivation of the inducing toxicant itself, and enzymes that repair or compensate for various types of damage caused by the toxicant

4.2 C × T ISSUES RELATED TO NATIONAL AMBIENT AIR QUALITY STANDARDS (ECO EFFECTS)

Allen Lefohn, ASL & Associates

In 1981, in discussing EPA's proposal to use long-term average concentration as the form of a standard to protect vegetation from the effects of ozone, a question arose about why all concentrations should be treated the same, as opposed to weighting differentially the higher, mid-level, and lower hourly average concentration. No evidence was cited in the literature to support this regarding growth reduction for crops. This began an area of research that has continued to the present.

One of the first questions was what types of ambient exposures are actually occurring? Figure 4-4 shows different types of exposure for ozone (same average and different distributions). If higher concentrations should be given greater weight than mid-level and lower, one has to be concerned about the episodic exposures and can't use average concentrations over time.

In 1996, EPA's conclusions concerning exposure indices for vegetation effects included the following:

- Exposure indices that weight the hourly ozone concentrations differentially appear to be the best candidates for relating exposure with predicted plant response.
- Peak-weighted, cumulative indices appear to have major advantages over the mean (e.g., 7-hr seasonal mean), peak indices (e.g., 2HDM) and the index that cumulates all hourly average concentrations (i.e., SUM00).
- OAQPS continues to believe that the selection of an appropriate integration time of interest should take into account the cumulative impact from repeated peaks over an entire growing season.

In 1982, another group performed an experiment assessing the importance of peaks (ambient versus uniform). They examined two exposure profiles: a 6-hr episodic profile of varying peak frequency, concentration, and duration, and a 6-hr profile of equivalent peak concentration and duration but constant concentration. They found that bean plants exposed to ozone with a simulated episodic ambient concentration distribution showed significantly more injury, less growth, and lower yield than those exposed to an equivalent dose of ozone with a uniform concentration distribution (Musselman et al. 1983. J. Amer. Hort. Sci. 108:347–351).

The EPA Corvallis group (Hogsett et al. 1985. Atmos. Environ. 19:1135–1145) compared a 30-day episodic profile of varying peak frequency, concentration, and duration, and a daily peak profile of equivalent peak concentration and duration each day. They found that with regard to expression of ozone exposure, the 7-hr means for these two profiles did not reflect the observed growth response. The episodic profile has the smaller seasonal means, yet caused greater yield reduction.

With regard to the SUM07 (the sum of all hourly average concentrations greater than or equal to 0.07 ppm) this group concluded that the same SUM07 exposure values for the two profiles did not reflect the observed growth response. The episodic profile caused greater yield reduction.

Given the problem that the higher concentrations elicited more of an adverse effect than the mid-level or lower concentration, over an ozone season (April-October), how does one cumulate the ozone exposure to come up with a metric that represents the potential impact? Figure 4-5 shows a sigmoidal weighting function.

Different Types of Exposures

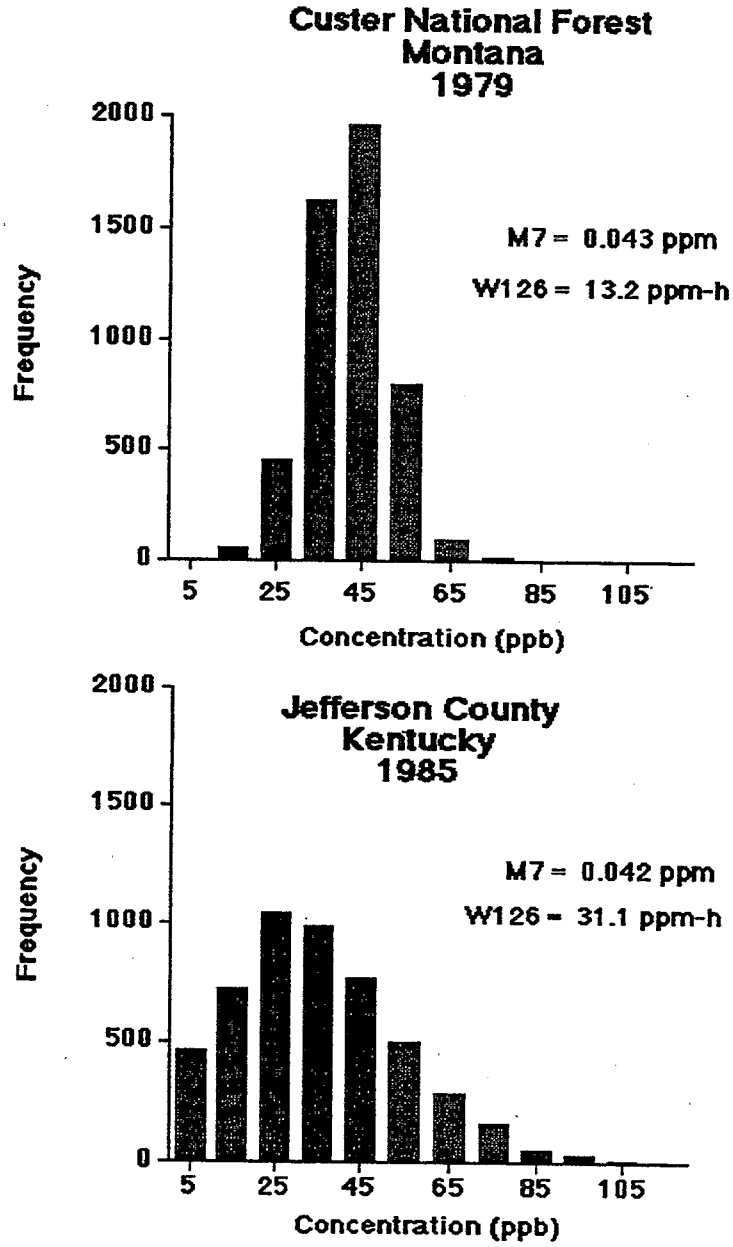


Figure 4-4

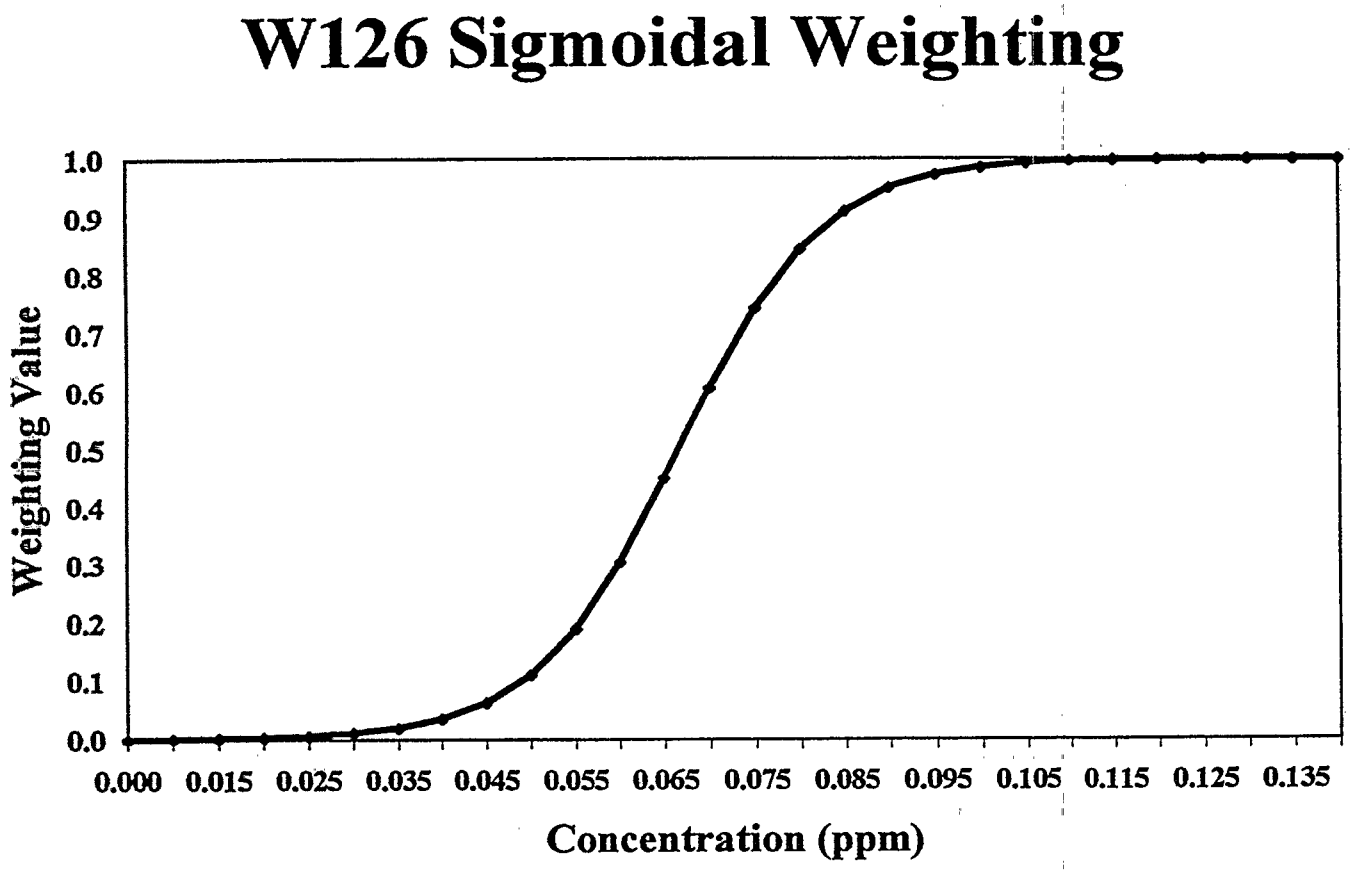

W126 Sigmoidal Weighting
Weighting Value
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.000
0.015
0.025
0.035
0.045
0.055
0.065
0.075
0.085
0.095
0.105
0.115
0.125
0.135
Concentration (ppm)

Figure 4-5

This W126 exposure index focuses on the higher hourly average concentrations, while retaining the mid- and lower-level values. To determine the W126 index, the sigmoidal weighting value at a specific concentration is multiplied by the concentration and then summed over all concentrations:

$$W_i = 1/[1 + M \times e^{-(A \times C_i)}]$$

where M and A are arbitrary positive constants (4403 and 126 ppm^{-1} respectively)
W_i = weighting factor for concentration C_i
C_i = concentration 1 in ppm

The design of the weighting function was based on:

- Truncating hourly average concentrations below 0.4 ppm
- Having an inflection point near 0.065 ppm
- Providing equal weighting of 1 for hourly average concentrations at approximately 0.10 ppm and above

Figure 4-6 shows how this index differs on an accumulation basis from the SUM00, which is directly linked to the average. The SUM00 spent too much time accumulating at the lower end of the distribution, and is not weighting the area of interest biologically (peaks).

On the health side, Hazucha et al. (1992, Am. Rev. Respir. Dis. 146:1487–493) found a greater effect for the triangular shape compared to the square wave (Figure 4-7), showing the relevance of the mathematics and developmental work on differential weighting. Hazucha et al.'s work illustrates the importance of the high concentrations with respect to the mid- and lower level values.

4.3 STATISTICAL MODELS FOR ASSESSING DOSE-RATE EFFECTS

Paige Williams, Harvard School of Public Health

This presentation addresses the following question: If we have data for different combinations of exposure duration and exposure levels, how do we extend the concept of a benchmark dose to account for duration of exposure?

4.3.1 Background

Use of a benchmark dose approach has become popular in risk assessment for non-cancer endpoints. The idea is to chose a dose-response model that reflects the probability of a toxic endpoint as a function of dose. Because these models have been adapted from cancer risk assessment, they are usually just a function of dose. For many non-cancer endpoints (e.g., in developmental toxicity studies), however, both the timing and the duration of exposure are crucial to the levels and even types of outcomes. Because regulatory agencies want to set exposure standards for varying lengths of exposure, the benchmark dose approach needs to be extended to incorporate duration and possibly timing of exposure.

Averages Versus W126

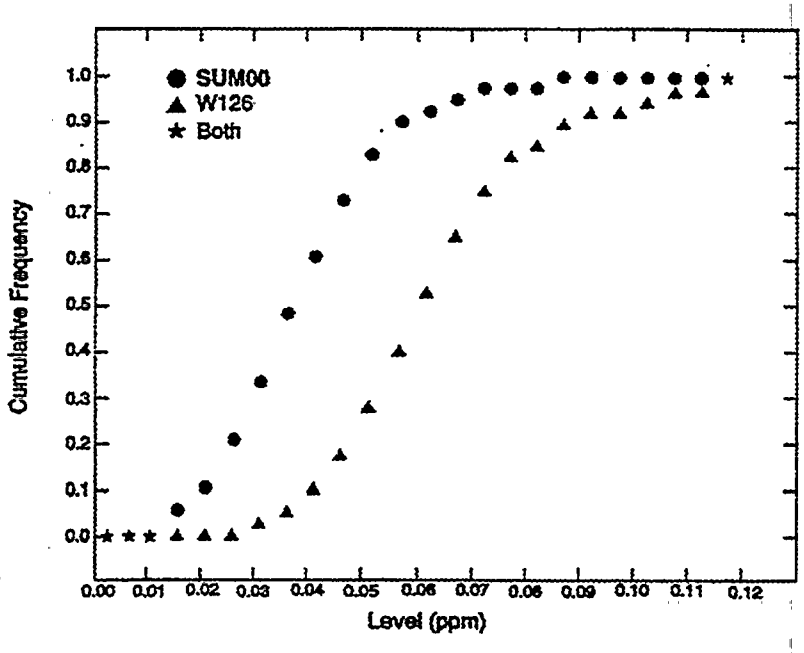
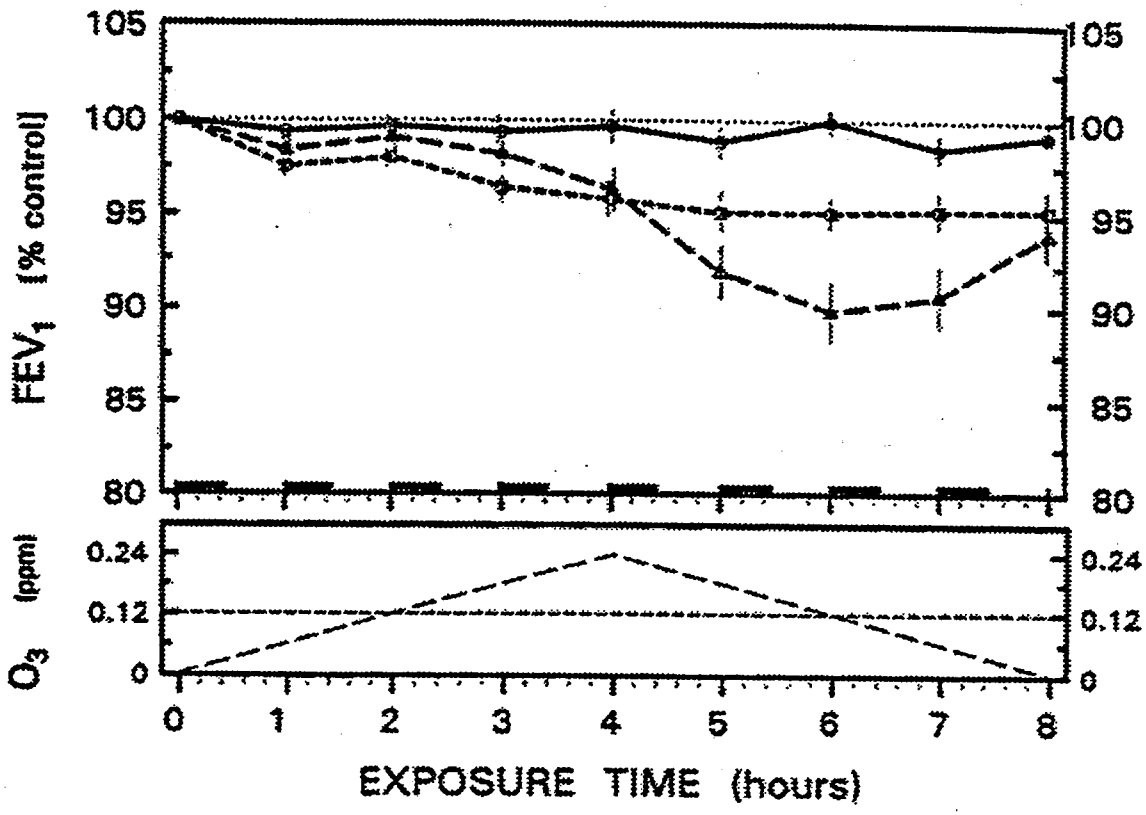


Figure 4-6

Square Wave Versus Triangle Exposure



Hazucha et al. 1992. *Am Rev Resp Dis* 146:1487-1493.

Figure 4-7

4.3.2 Ethylene Oxide Study

The goals of this study were to:

- Assess effects of EtO on developmental toxicity
- Evaluate applicability of Haber's Law
- Develop risk assessment methods for dose-rate studies
- Improve approaches for designing dose-rate studies

The study design included three multiples of C × T; within these three multiples (control, 2100 ppm hours, and 2700 ppm hours) the researchers looked at various combinations of C × T that gave the same overall cumulative exposure. This allowed an assessment of any departures from Haber's law, e.g., the percentage of deaths was much higher for short acute than longer chronic exposures, for the same C × T value.

Figure 4-8 shows the four steps usually used for calculating a benchmark dose (without regard to duration). The two methods for computing the lower confidence limit are shown in Figure 4-9. Figure 4-10 shows the four steps for extending these models with information on combinations of dose levels and duration of exposure.

The extended models fit better than Haber's model; the coefficients for time were always negative, indicating that short acute exposures were causing more damage than longer chronic exposures (Figures 4-11 to 4-13).

Calculating the effective dose/duration contour is described in Figure 4-14. Figure 4-15 shows that the extended model allows for extra effects of duration in addition to cumulative exposure. At very high levels of exposure, the allowable duration is much shorter for the same level of toxicity than under Haber's model.

To calculate the lower confidence contours, either the lower effective dose or benchmark dose approach can be extended to account for duration of exposure (Figures 4-16 and 4-17). One problem with this is the simultaneous inference problem, described in Figure 4-18.

A final topic is accounting for multiple outcomes found in many non-cancer studies. Figures 4-19 and 4-20 outline an approach that takes into account fetal death and fetal malformation and extending that to a situation where both dose and duration information is available. With multiple outcomes *and* multiple covariates, one has to solve for the effective dose iteratively by computer. The extended dose/duration contours (Figure 4-21) show the need to be much more conservative in the risk assessment than just choosing the most sensitive endpoint.

Other applications have included ordinal outcomes, continuous outcomes, and joint assessment of discrete and continuous outcomes. Further research needs include deriving lower confidence contours on EDDC and issues of optimal study design.

Risk Assessment for Non-Cancer Endpoints
Benchmark Dose Approach

- (1) **Choose a dose-response model**

Logit: $P(D) = \frac{1}{1+exp[-(\alpha+\beta D)]}$

Probit: $P(D) = \Phi(\alpha + \beta D)$

In General: $P(D) = \mathcal{F}(\alpha + \beta D)$

- (2) **Fit model**, using GEE's if necessary to account for litter effect and/or correlation between multiple responses

- (3) **Estimate effective dose (ED)** - the dose which results in a specified increase in risk over background.

Excess Risk Functions:

$$\text{Additive:} \quad r(D) = P(D) - P(0)$$

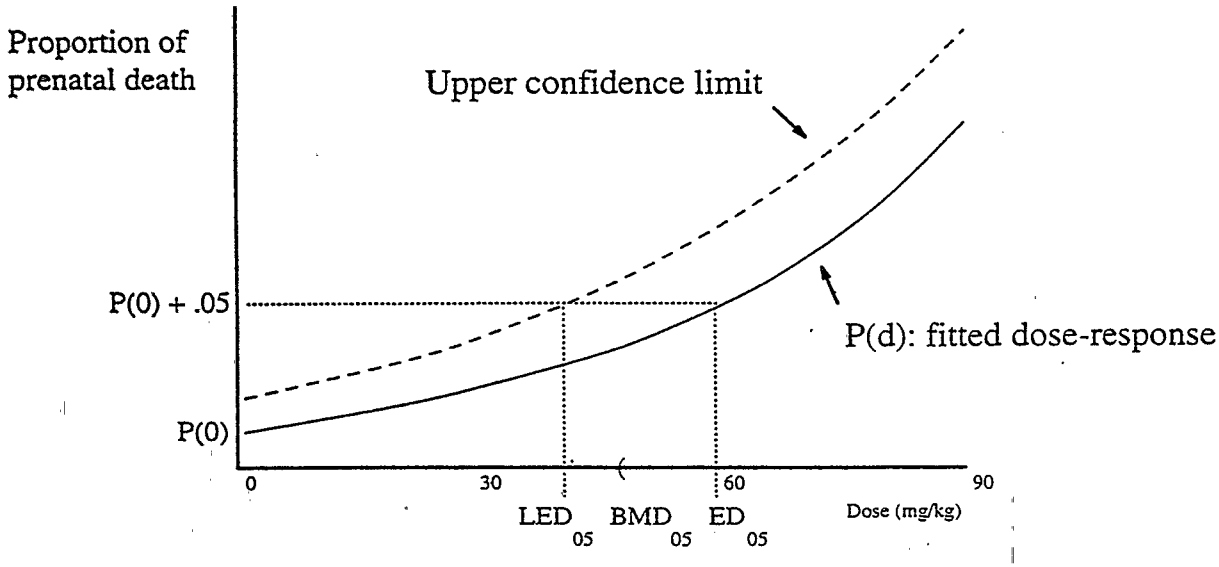
$$\text{Relative:} \quad r(D) = \frac{P(D) - P(0)}{1 - P(0)}$$

Ex: For a 5% excess risk of an adverse response (ED_{05}), $r(D) = 0.05$.

- (4) **Compute lower confidence limit** on effective dose to get the **benchmark dose** (also referred to as BMDL)

Figure 4-8

Computing the Lower Confidence Limit



Two Methods:

- **LED** - Lower Effective Dose
  estimate the variability of the predicted dose-response function to calculate upper confidence limit on the entire curve, then find dose on upper confidence limit curve corresponding to the excess risk.

- **BMD** - Benchmark Dose
  estimate the variability of the predicted dose ED (eg. ED10), and calculate a lower confidence limit (proposed by Crump, 1984). One potential problem is that it is possible to get negative values for the BMD.

Figure 4-9

Dose Response Models for Dose-rate Studies

- (1) **Dose-response models** - a function of exposure level (D) and duration of exposure (T).

Haber's Model:

$$\text{logit}(P(D,T)) = \beta_0 + \beta_3 \, D * T$$

Extended Model:

$$\text{logit}(P(D,T)) = \beta_0 + \beta_1 D + \beta_2 T + \beta_3 \, D * T$$

- (2) **Fit models to get "response surface":**
If necessary, use GEEs to account for litter effects

- (3) **Effective dose/duration contour** -
all possible combinations of D and T that jointly lead to a certain increase in risk over background.

Additive Excess Risk Function:

$$r(D,T) = P(D,T) - P(0,0)$$

- (4) **Calculate the "lower confidence contour"** - i.e., the lower confidence limit on the effective dose-duration contour

Figure 4-10

Example: Ethylene Oxide Study
Dose Response Models estimated using GEEs:

MATERNAL MORTALITY:

- **Haber's Model**
  $logit[P(D,T)] = -2.02 + 4.03\text{x}10^{-4}\, D * T$

- **Extended Model**
  $logit[P(D,T)] = -1.08 + -0.36\, T + 5.97\text{x}10^{-4}\, D * T$

- **Constrained Model**
  $logit[P(D,T)] = -1.99 + -0.48\, T\delta_D + 11.6\text{x}10^{-4}\, D * T$

FETAL DEATH:

- **Haber's Model**
  $logit[P(D,T)] = -1.99 + 3.88\text{x}10^{-4}\, D * T$

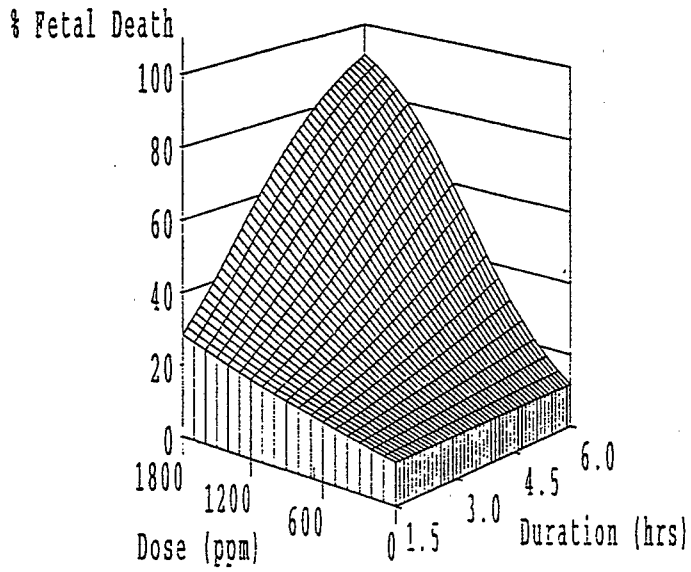
- **Extended Model**
  $logit[P(D,T)] = -1.18 + -0.31\, T + 5.64\text{x}10^{-4}\, D * T$

- **Constrained Model**
  $logit[P(D,T)] = -1.99 + -0.48\, T\delta_D + 11.6\text{x}10^{-4}\, D * T$

Figure 4-11

Ex: Ethylene Oxide Study Fitted Response Surfaces for Fetal Death under Haber's Model and Extended Model

Habers Model



Extended Model

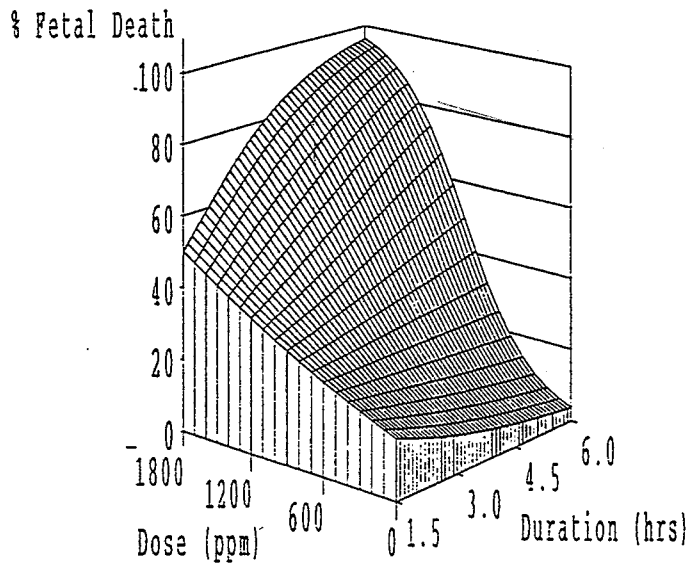
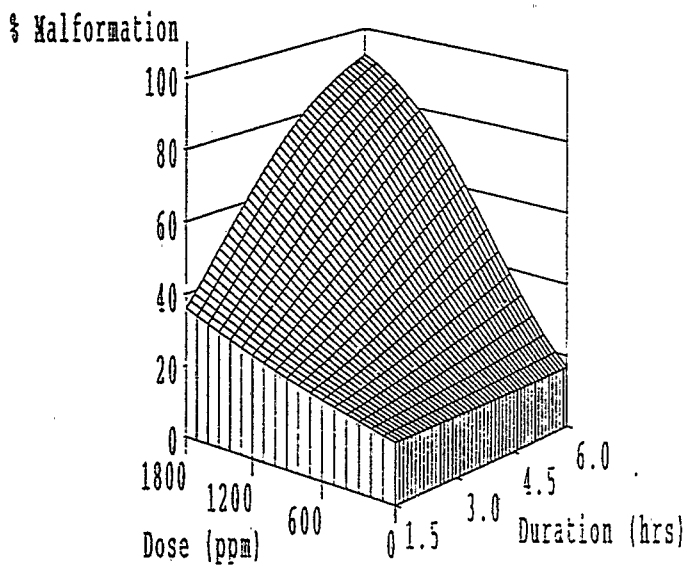


Figure 4-12

Ex: Ethylene Oxide Study
Fitted Response Surfaces for Fetal Malformation under Haber's Model and Extended Model

Habers Model



Extended Model

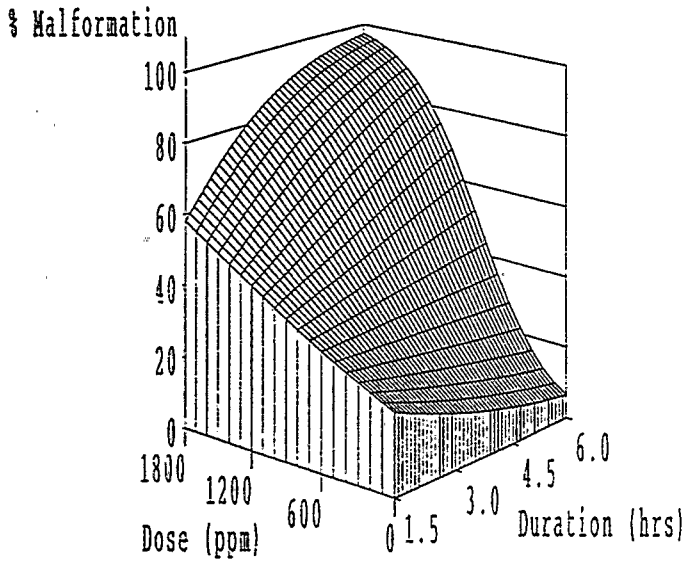


Figure 4-13

Calculating the Effective Dose/Duration Contour

In general, the excess risk function is

$$\begin{aligned} r(D,T) &= P(D,T) - P(0,0) \\ &= \mathcal{F}(\beta_0 + \beta_1 D + \beta_2 T + \beta_3 D * T) - \mathcal{F}(\beta_0) \end{aligned}$$

and the effective dose/duration contour is the set of all points (D,T) in the design space such that $r(D,T) = \gamma$

$$C : \{(D,T) : r(D,T) = \gamma, \quad D \geq 0, T \geq 0\}$$

By fixing one of the two variables, we can solve for the other to get all points on this contour (**isobol**); i.e., for a given T:

$$D(T) = \frac{\mathcal{F}^{-1}(\gamma + \mathcal{F}(\beta_0)) - (\beta_0 + \beta_2 T)}{\beta_1 + \beta_3 T}, \quad (D,T) \in \mathrm{R}^+$$

True contour: $D(T, \beta, \gamma)$ **Estimated contour:** $D(T, \hat{\beta}, \gamma)$

Figure 4-14

Effective Dose-Duration Contours for Fetal Death under Haber's Model and Extended Model

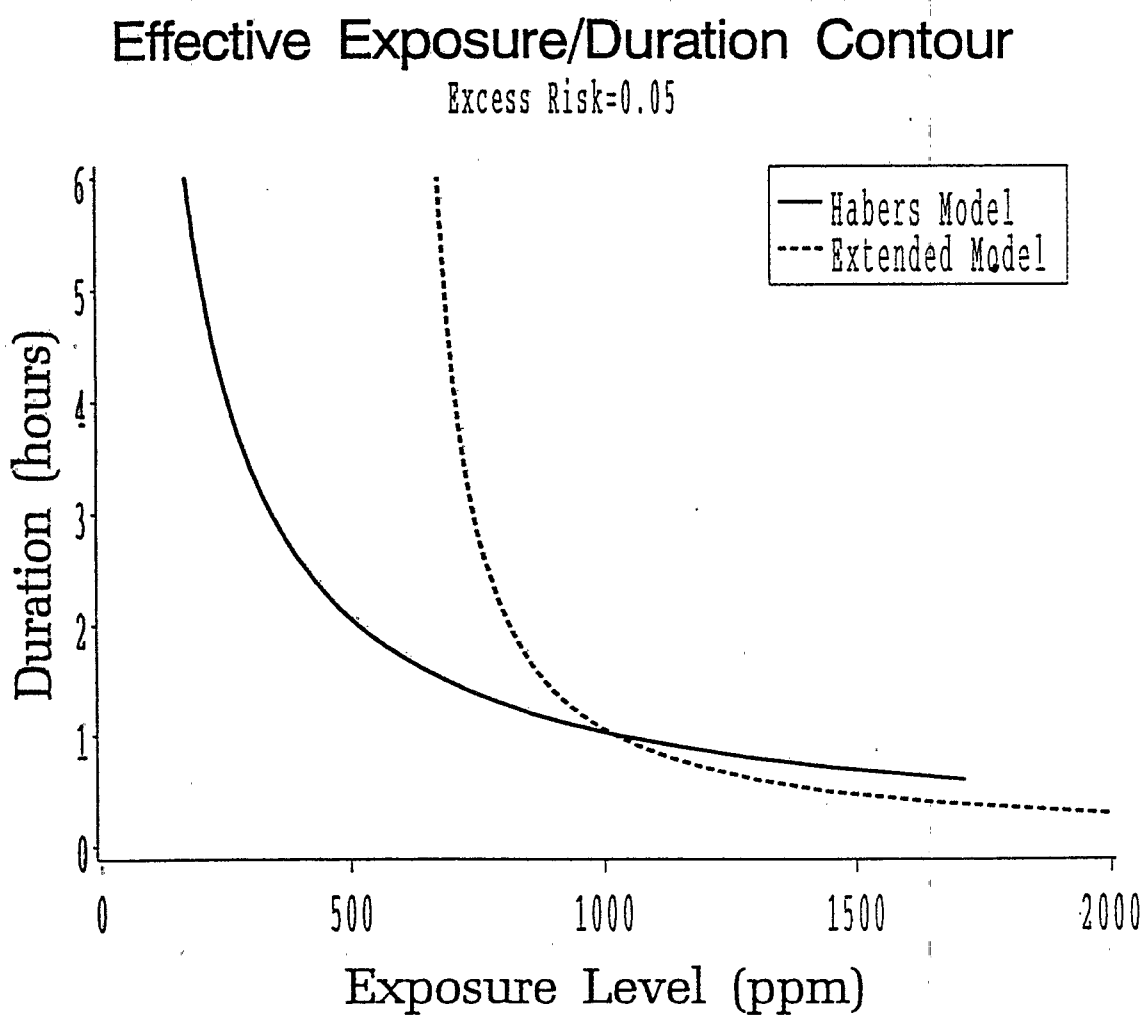


Figure 4-15

Calculation of Lower Confidence Contours for Combinations of Dose and Duration

Both the LED and the BMD approaches can be extended to account for duration of exposure.

I. Extending the LED approach:

Find the upper 95% limit on the dose-duration response surface, and then determine where this surface intersects the γ-level increase in risk.

II. Extending the Benchmark Dose approach:

Fix D, and calculate the standard error of the estimated time point T on the contour using the delta method

$$\hat{\sigma}_T = \left(\frac{\partial T(D,\hat{\beta},\gamma)}{\partial \beta}\right)^T \hat{\Sigma}_\beta \left(\frac{\partial T(D,\hat{\beta},\gamma)}{\partial \beta}\right)\Bigg|_{\beta=\hat{\beta}}$$

Repeat this for many values of D to get the lower contour:

$$LC_{\text{fix D}} : \left\{(D,T) : T(D,\hat{\beta},\gamma) - z\hat{\sigma}_T\right\}$$

(Or fix time, and calculate the standard error of the estimated dose D on effective dose/duration contour.)

Figure 4-16

Lower Effective Contours (LEC's) using "fixed time" approach

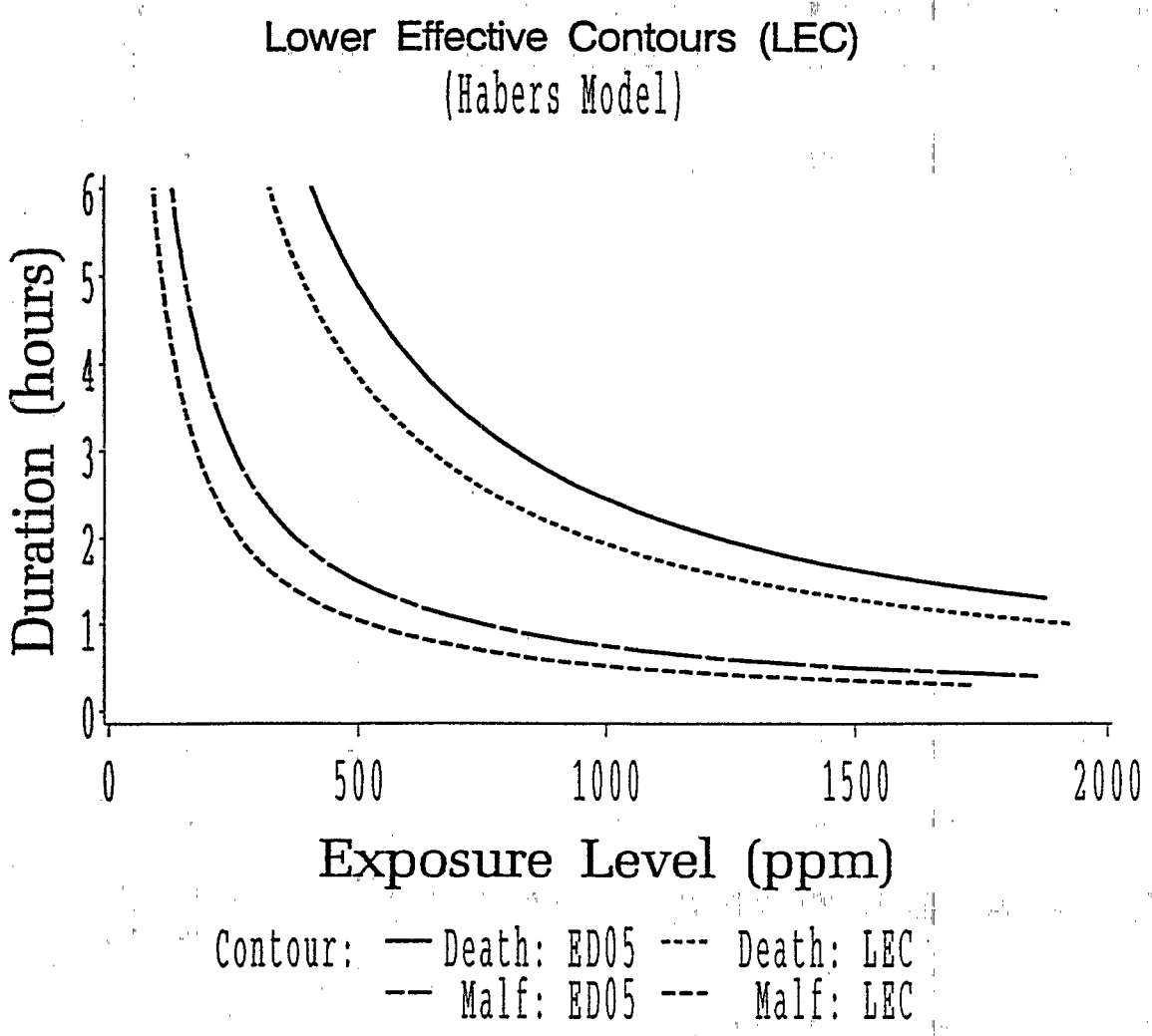


Figure 4-17

Simultaneous Inference Problem

Usually we set z=1.645 for a one-sided 95% confidence interval.

However, the coverage probability of the lower confidence contour must correspond to the *entire* true contour, and not just to a single point.

Using z=1.645 will give coverage probabilities <u>under</u> 95%.
This was confirmed in simulation studies:

Type of Lower Confidence Contour	Mean Crossing Rate	Standard Error
Lower Effective Contour	19.2%	0.7%
Benchmark Fixed-Dose	14.6%	0.7%
Benchmark Fixed-Time	5.1%	0.4%

How do we choose the correct critical value, z?

An approximation has been developed using Hotellings' Theorem on the volume of tubes (Johansen and Johnstone, 1990):

$$\alpha \approx \int_z^\infty \left[\frac{|v(t)|}{2\pi} \left(1 - \frac{z}{w}^2\right)^{1/2} + \frac{1}{2} \Pr\left(Beta(\frac{1}{2}, 1) \geq \left(\frac{z}{w}\right)^2 \right) \right] \cdot \Pr(\sqrt{\chi^2_{(3)}} \in dw)$$

This approximation can be used to:

- estimate z for a particular dataset
- estimate true Type I error (α) for a given z

Figure 4-18

Accounting for Dose-rate Effects on Multiple Developmental Outcomes

Ryan (92) considered the hierarchical endpoints:

- **fetal death**
- **fetal malformation**

If we define probabilities as a function of dose, D:

$$P_{FD}(D) = \Pr(\text{fetal death})$$

$$P_{FM}(D) = \Pr(\text{malformation}|\text{no fetal death})$$

then Ryan showed that the overall probability of an abnormal event can be written as:

$$P_{abn}(D) = 1 - [1 - P_{FM}(D)]\,[1 - P_{FD}(D)]$$

This same principle can be applied to assess dose-rate effects on abnormal outcomes, as follows:

$$P_{abn}(D,T) = 1 - [1 - P_{FM}(D,T)]\,[1 - P_{FD}(D,T)]$$

Figure 4-19

Effective Dose-Duration Contours with Multiple Developmental Outcomes

Single Outcome, Single Covariate:

$$P(d) = \mathcal{F}(\alpha + \beta d)$$

$$r(d) = P(d) - P(0) = \mathcal{F}(\alpha + \beta d) - \mathcal{F}(\alpha)$$

$$\widehat{ED}_\gamma = \frac{\mathcal{F}^{-1}(\gamma + \mathcal{F}(\hat{\alpha})) - \hat{\alpha}}{\hat{\beta}}$$

Single Outcome, Multiple Covariates:

$$P(d,t) = \mathcal{F}(\alpha + \beta_1 t + \beta_2 d * t)$$

$$r(d,t) = P(d,t) - P(0,0) = \mathcal{F}(\alpha + \beta_1 t + \beta_2 d * t) - \mathcal{F}(\alpha)$$

$$\widehat{ED}_\gamma(t) = \frac{\mathcal{F}^{-1}[\gamma + \mathcal{F}(\hat{\alpha})] - (\hat{\alpha} + \hat{\beta}_1 t)}{\hat{\beta}_2 t}$$

Multiple Outcomes, Multiple Covariates:

$$P(d,t) = 1 - [1 - P_{FM}(d,t)]\,[1 - P_{FD}(d,t)]$$

$$\begin{aligned} r(d,t) &= P(d,t) - P(0,0) \\ &= [\mathcal{F}_D(\alpha_d + \beta_{1d} t + \beta_{2d} d * t) - \mathcal{F}_D(\alpha_d)] \\ &\quad + [\mathcal{F}_M(\alpha_m + \beta_{1m} t + \beta_{2m} d * t) - \mathcal{F}_M(\alpha_m)] \\ &\quad - [\mathcal{F}_D(\alpha_d + \beta_{1d} t + \beta_{2d} d * t)\,\mathcal{F}_M(\alpha_m + \beta_{1m} t + \beta_{2m} d * t) \\ &\quad - \mathcal{F}_D(\alpha_d)\,\mathcal{F}_M(\alpha_m)] \end{aligned}$$

Effective dose cannot be expressed analytically, but can be calculated using an iterative approach.

Figure 4-20

Ethylene Oxide Study

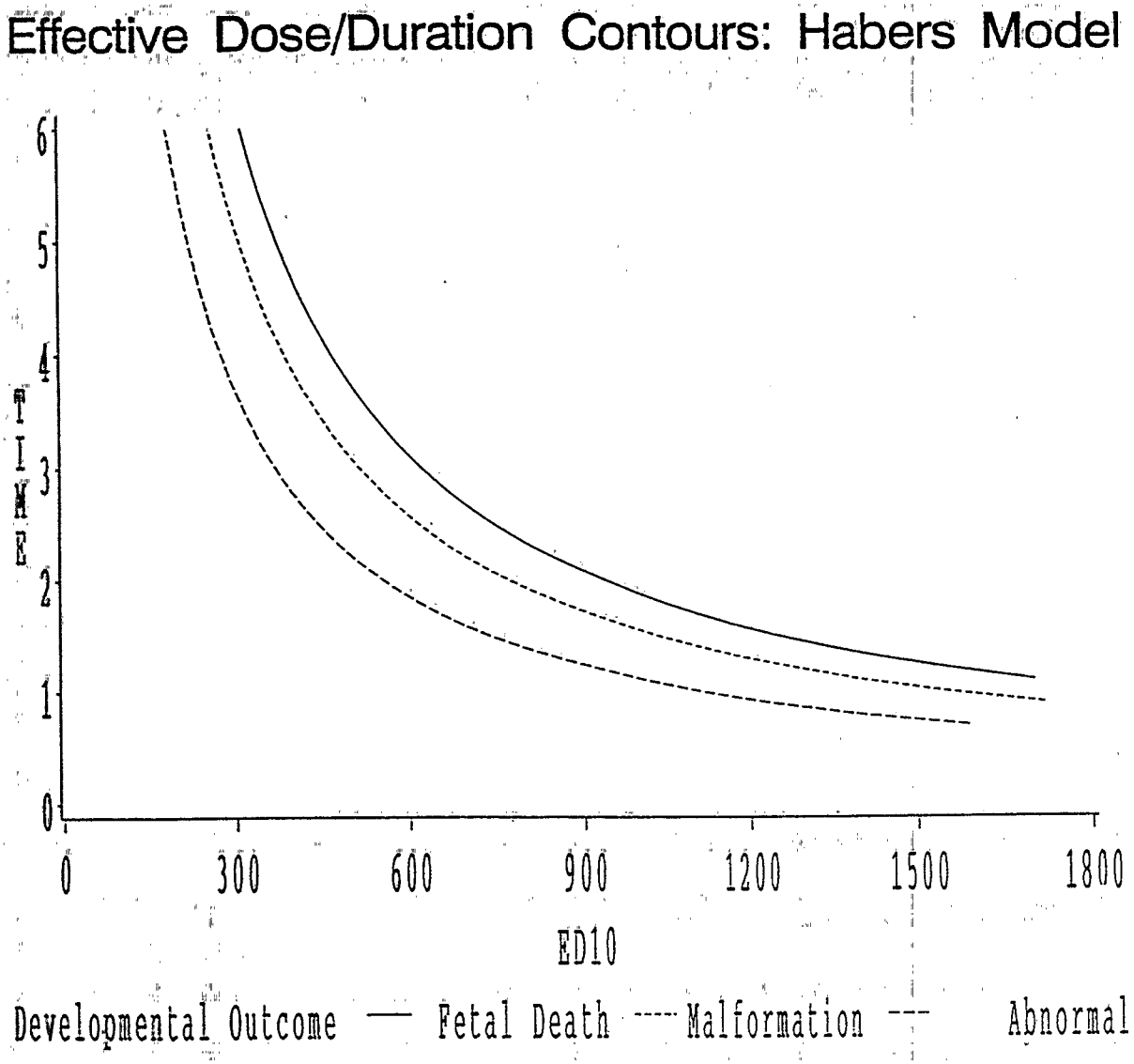


Figure 4-21

SECTION FIVE
PLENARY PRESENTATIONS:
DOSIMETRY AND MECHANISTIC MODELING

5.1 DOSIMETRY: MECHANISTIC DETERMINANTS OF EXPOSURE-DOSE-RESPONSE

Annie Jarabek, EPA National Center for Environmental Assessment

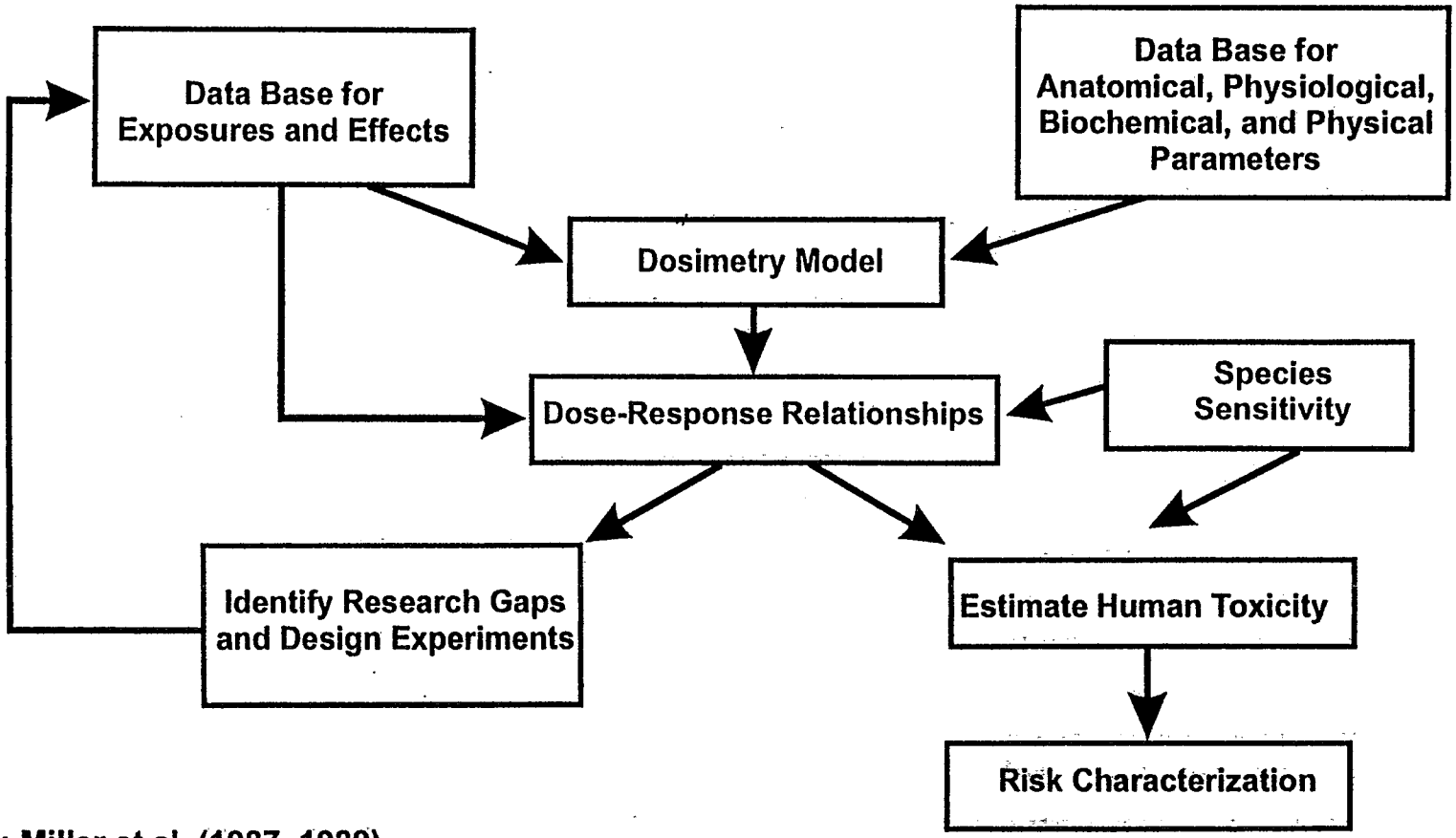
Often we don't have the database on the anatomical, physiological, and biochemical parameters needed to do a truly comprehensive and mechanistically based dosimetry model, so we are often looking at correlations or empirical descriptions of processes we think are important. The dosimetry model is central (Figure 5-1) to in informing the dose-response model. This dosimetry modeling approach is iterative and often points to additional data that might be needed and thus can be used for experimental design. Case studies with particles and gases will be presented to illustrate the approach.

Figure 5-2 presents another way to look at the exposure-dose-response schema, as presented in Section Two, and identifies parameters and processes familiar to experimentalist. Note that defense mechanisms are described at various levels of organization from molecular to whole organ. The pharmacokinetics component needs to be linked to the pharmacodynamic component. With respect to the vinyl acetate model discussed previously, it was generally accepted that acetic acid was the important metabolic product of metabolism, but one could say that the pH could be the response or a dosimeter; clearly more insight is needed in the pharmacodynamic arena.

Figure 5-3 shows the HEC default approach for particles. The fractional deposition is important because the same aerosol distribution will deposit in a human versus a rat in a much different fashion (Figure 5-4). The deposited fraction is an integration of deposition efficiency and inhalability. In rats, the deposition fraction is about .45 whereas it is almost 1.0 in humans. This has important implications for interspecies extrapolation and biologic plausibility. For example, rats may have to be exposed to much higher concentrations in order to get an inhaled dose sufficient to manifest toxicity. The model currently used for upper respiratory tract model in the rat is empirical, as is the International Commission on Radiation Protection dosimetry model for humans.

Figures 5-5 through 5-7 show how mechanistic models can start to impart important nonlinearities (e.g., nonlinear pattern of the deposition fraction) that might not be considered when just looking at the exposure. Note the influence of activity pattern on ventilation rate or differences in environmental aerosol particle distributions (Phoenix versus Philadelphia) on the resultant mass deposition fraction in each region. Mass deposition fraction may be an appropriate metric to characterize acute effects, but differences can be introduced by how it is normalized. Figures 5-8 and 5-9 show mass of particles deposited per surface area or mass normalized to lung tissue. Figure 5-10 shows particle number fraction deposited versus aerodynamic diameter, a different relationship than shown with mass. Mechanistic insight needs to help inform the choice of these various dose metrics. For chronic endpoints, we need to incorporate additional parameters in the model, i.e. those that characterize clearance, to characterize retained dose (Figure 5-11). The simulation showed the importance of getting a handle on the clearance parameters, and that one of the key uncertainties of the model is the dissolution-absorption rate.

Integrated Dosimetry Modeling Approach for Characterizing Risk



Source: Miller et al. (1987, 1989)

Figure 5-1

Species- and Chemical-Specific Influences on Exposure-Response Relationships

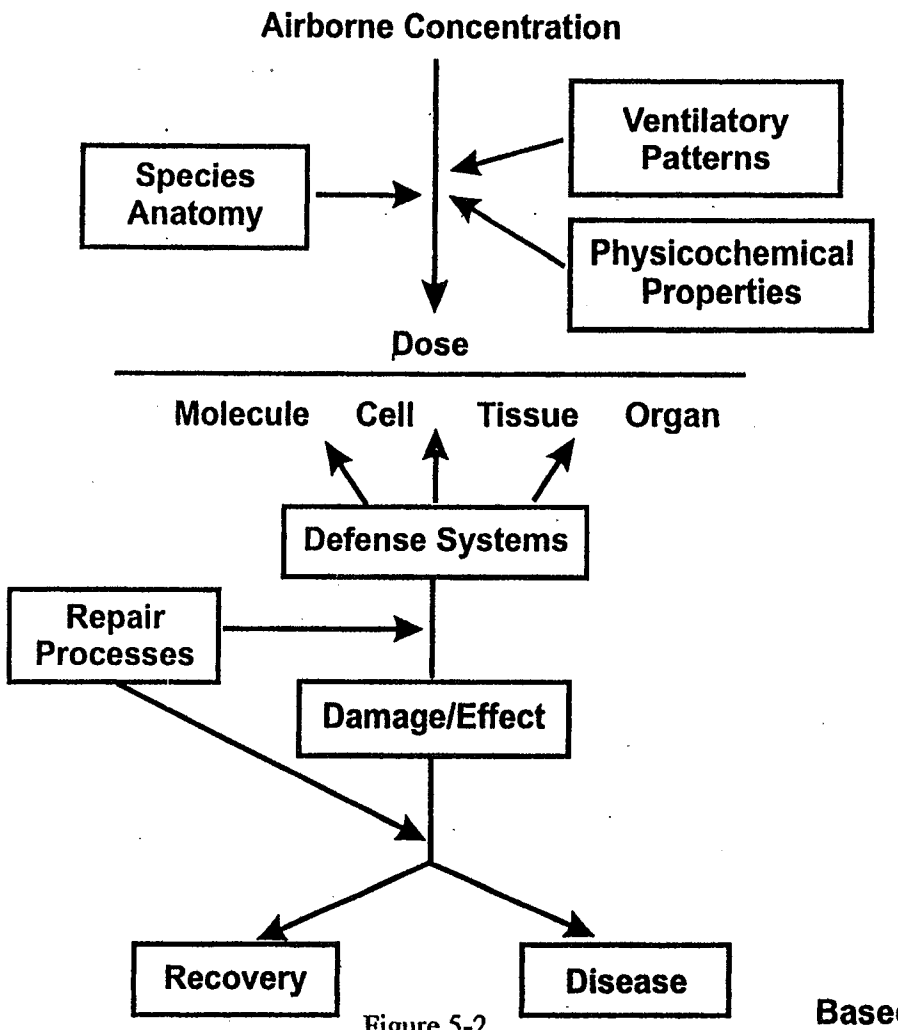


Figure 5-2

Based on: Martonen & Miller (1986)

HEC Default Approach Particles

$$NOAEL\ (HEC) = NOAEL(ADJ) \times RDDR_r$$

$$RDDR_r = RDDR(A) / RDD(H) =$$

$$NF(H) / NF(A) \times VE(A) / VE(H) \times F_r(A) / F_r(H)$$

R = Region of observed toxicity (ET, TB, PU, TH, TOTAL) for extrapolation.
NF = Normalizing Factor. SA for respiratory tract effects and BW for remote

Figure 5-3

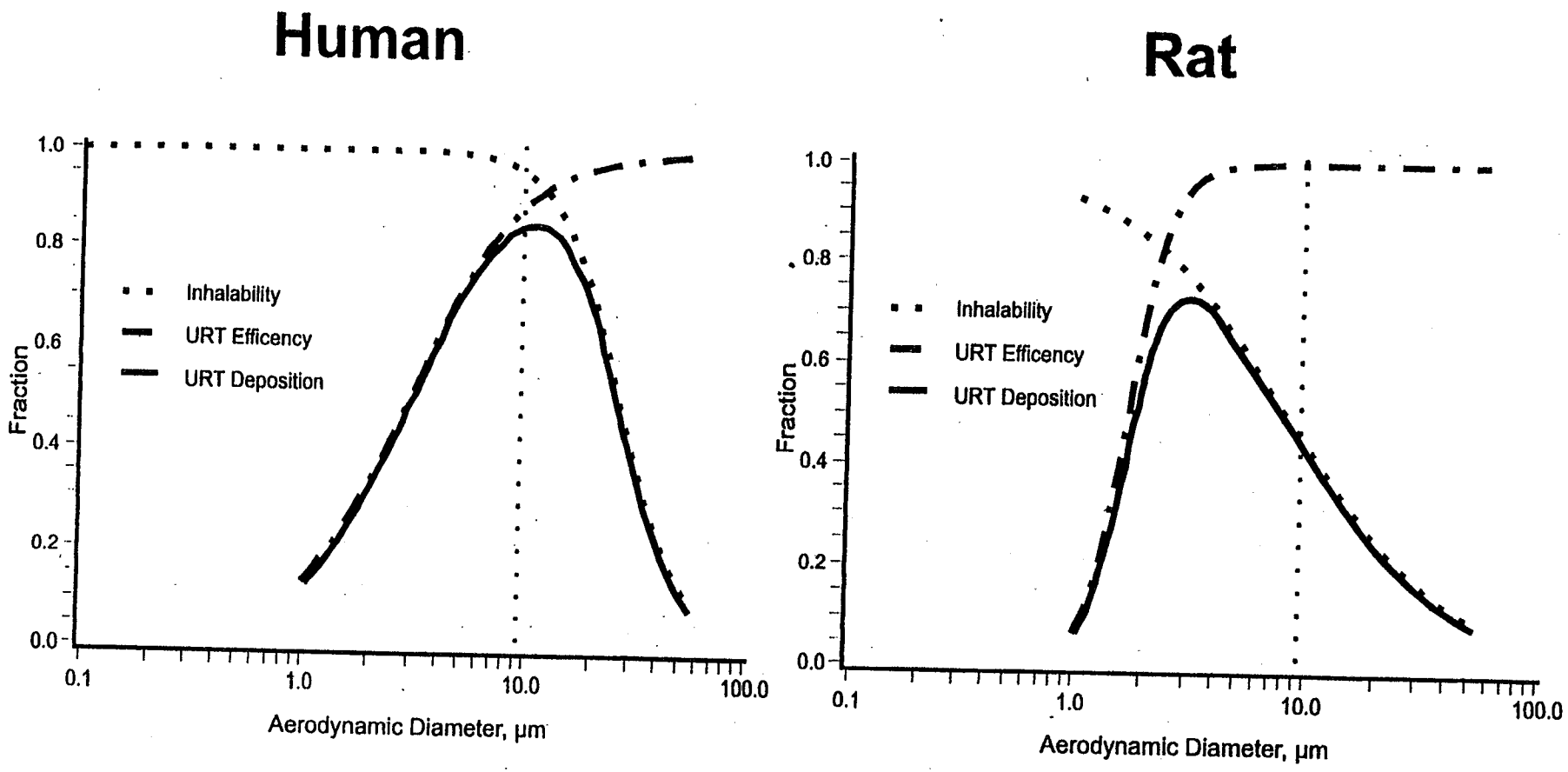

Human
Rat
Inhalability
URT Efficency
URT Deposition
Fraction
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1.0
10.0
100.0
Aerodynamic Diameter, µm

Figure 5-4

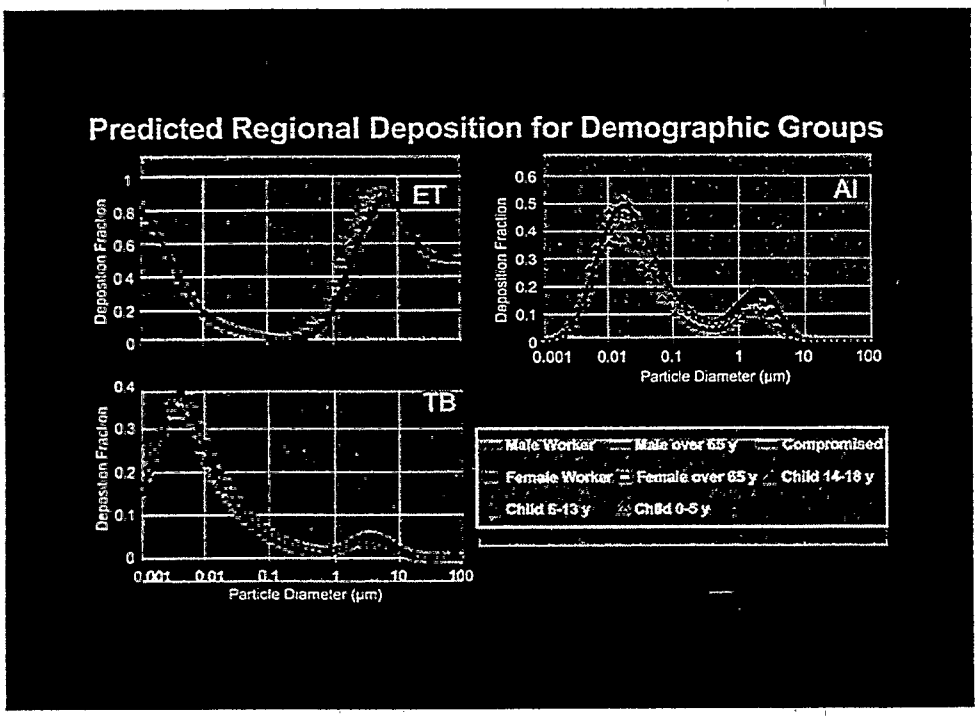

Predicted Regional Deposition for Demographic Groups
ET
AI
TB
Deposition Fraction
Particle Diameter (µm)
Male Worker
Male over 65 y
Compromised
Female Worker
Female over 65 y
Child 14-18 y
Child 6-13 y
Child 0-5 y

Figure 5-5

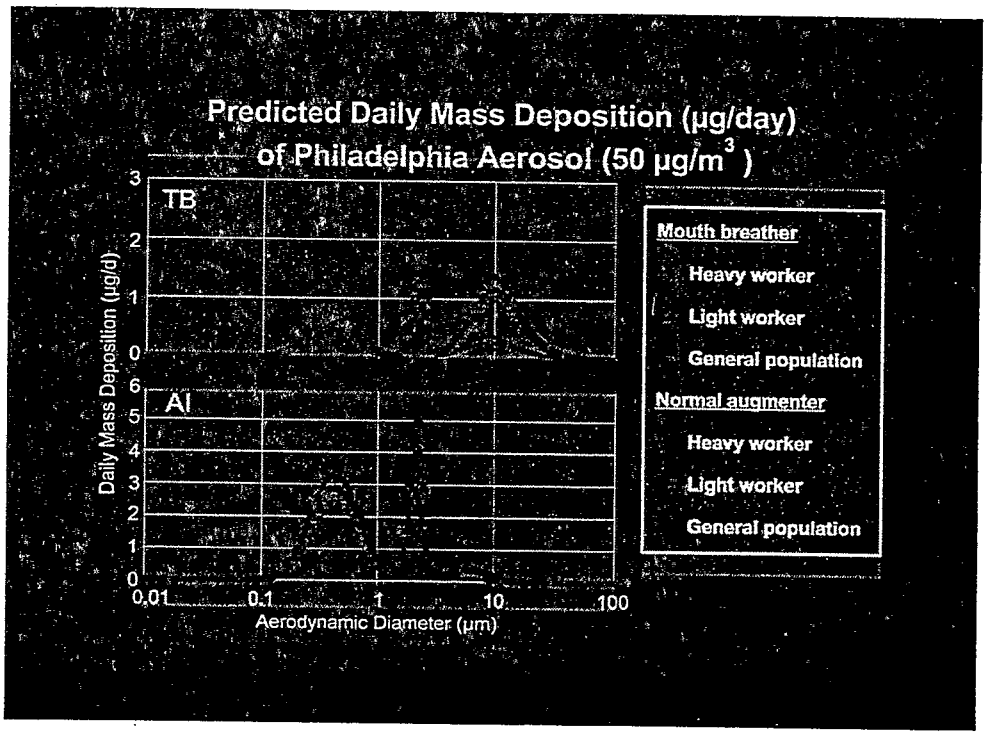


Figure 5-6

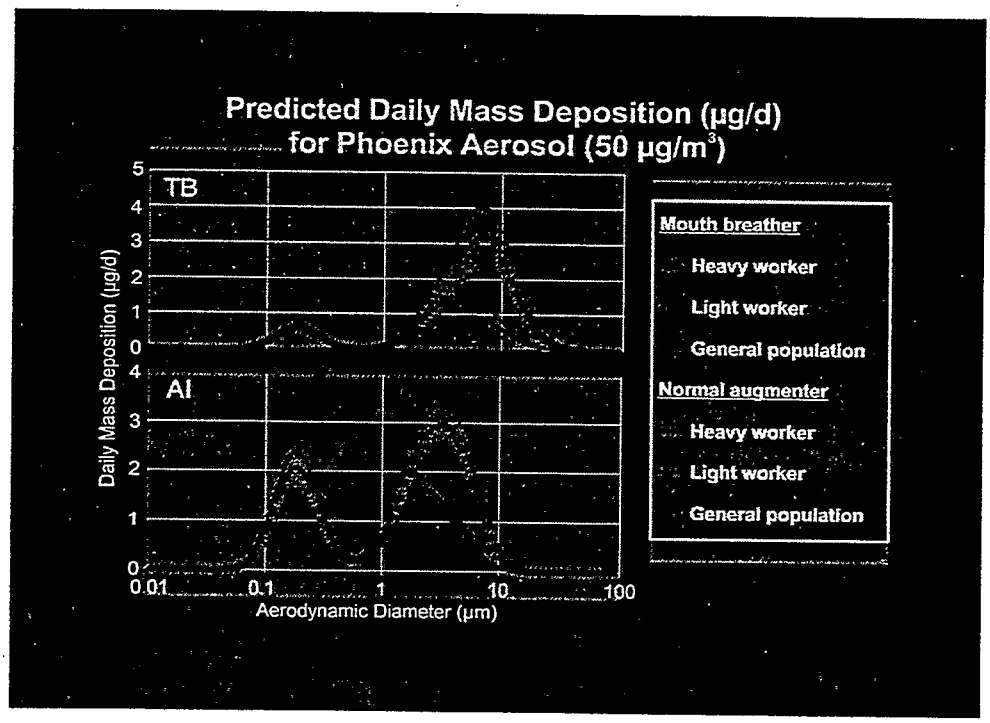


Figure 5-7

Predicted Daily Mass of Particles Deposited Per Unit of Tissue Surface Area (ng Particles/cm^2/Day) in a Normal Augmenter for Aerosols Inhaled for 24 Hours at 50 µg/m^3

	Philadelphia			Phoenix		
Region[a]	Fine	Intermodal	Coarse	Fine	Intermodal	Coarse
Extrathoracic (ET)	81	96	560	16	140	890
Tracheobronchial (TB)	3.2	1.3	0.9	3.0	1.8	3.5
Alveolar-Interstitial (AI)	0.025	0.0077	0.00078	0.018	0.012	0.0081

[a] Surface area of ET epithelium = 470 cm^2; TB epithelium = 2690 cm^2; AI epithelium = 1.475x10^6 cm^2

Figure 5-8

Predicted Daily Mass of Particles Deposited Per Unit of Tissue Mass (ng particles/gram tissue/day) In a Normal Augmenter for 24 Hours/Day at 50 µg/m³

	Philadelphia			Phoenix		
Region[a]	Fine	Intermodal	Coarse	Fine	Intermodal	Coarse
Extrathoracic (ET)	16,000	19,000	110,000	3,200	28,000	180,000
Tracheobronchial (TB)	1,700	670	470	1,500	920	1,800
Alveolar-Interstitial (AI)	34	10	1	24	16	11

[a] Mass of ET epithelium = 2.4 g, calculated from surface area of 470 cm^2 x average thickness of 50 µm; TB epithelium = 5.2 g, calculated from surface area of 290 cm^2 for the BB epithelium x average thickness of 15 µm, plus surface area of 2400 cm^2 x average thickness of 15 µm; AI epithelium = 1100 g

Figure 5-9

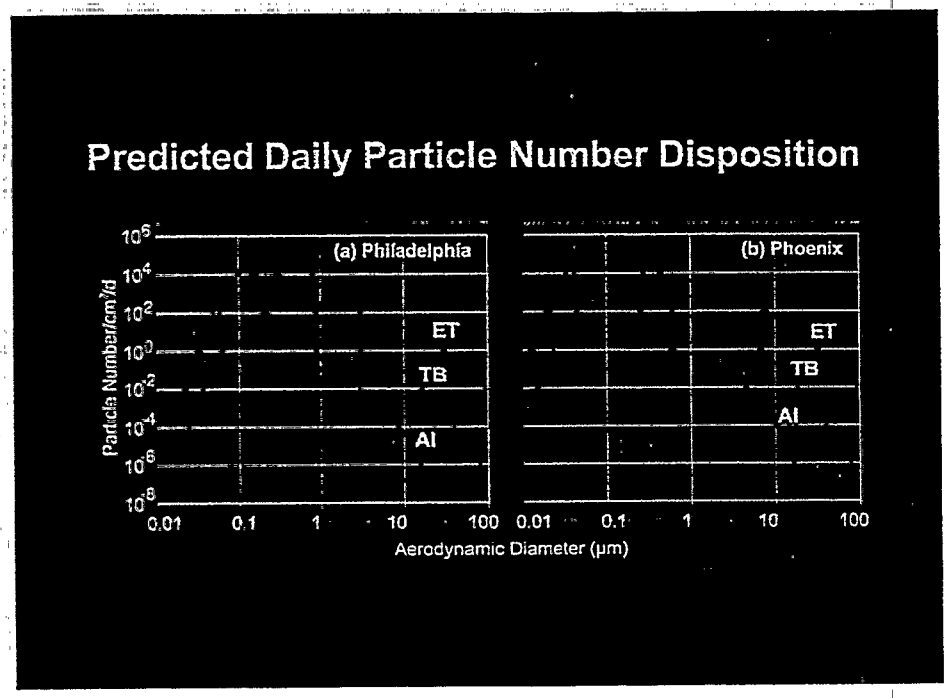


Figure 5-10

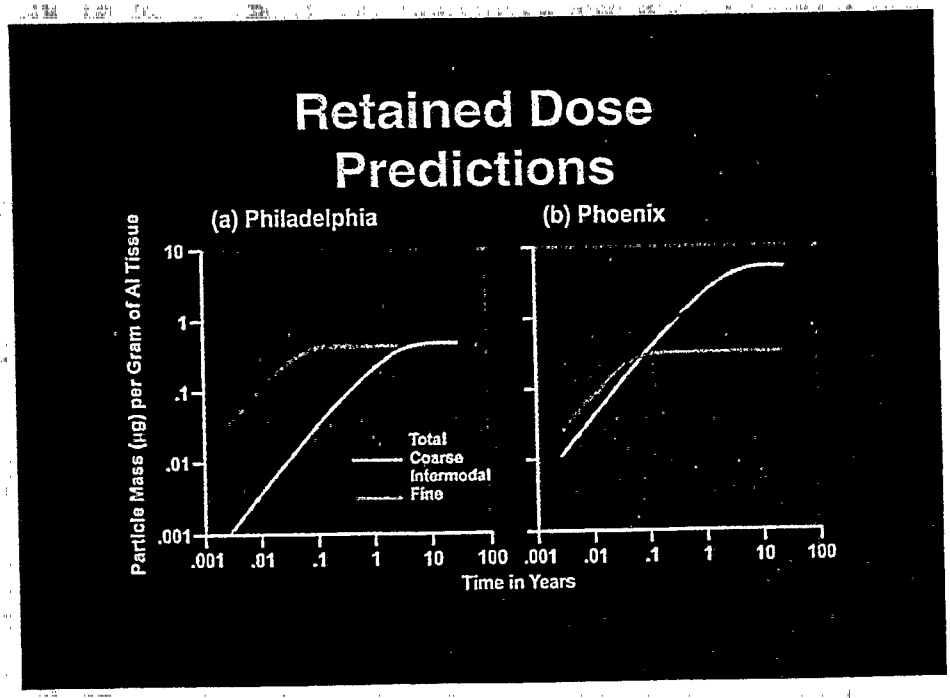


Figure 5-11

To illustrate similar considerations for gases, a simulation exercise was conducted exploring C × T relationships for dichloromethane (DCM) and perchloroethylene (PERC). These two were chosen because they differ in both key physicochemical parameters (e.g., fat-blood partition coefficients of 6.19 versus 121.0 for DCM versus PERC) and metabolic parameters (e.g., V_{max} of 11.54 versus 0.18 for DCM versus PERC) that would likely influence dose-duration relationships of different metrics. Figures 5-12 to 5-16 illustrate output for seven different simulations that have an equivalent C × T exposure product. For example, a 0.5-hr exposure at 400 ppm, a 4-hr exposure at 50 ppm, and an 8-hr exposure at 25 ppm are simulations that have an equivalent C × T exposure product of 200 ppm-hr. If "Haber's law" held then the plot of C × T products versus T would be a straight horizontal line.

Mechanistic modeling will inform default approaches in the future by serving as templates to determine the key processes and parameters and influence the next generation of dosimetry modeling.

The advantages of the PBPK/dosimetry modeling approach include the following:

- It allows integration and extrapolation using diverse data.
- It predicts complex kinetic behavior.
- It has the capability to "lump" or "split" model structure to explore dose-response.
- It enables interspecies dosimetric comparisons.
- It allows parameter scaling across species.
- It facilitates hypothesis generation.
- It identifies needed areas of research.

5.2 DOSIMETRY AND MECHANISTIC MODELING

Harvey Clewell, ICF Kaiser

The role of mechanistic modeling is to define the relationship between external concentration or dose and an internal measure of biologically effective exposure in both the experimental animal and the human. Tissue dose equivalence is an underlying assumption of dosimetry in risk assessment:

- Effects occur as a result of tissue exposure to the toxic form of the chemical.

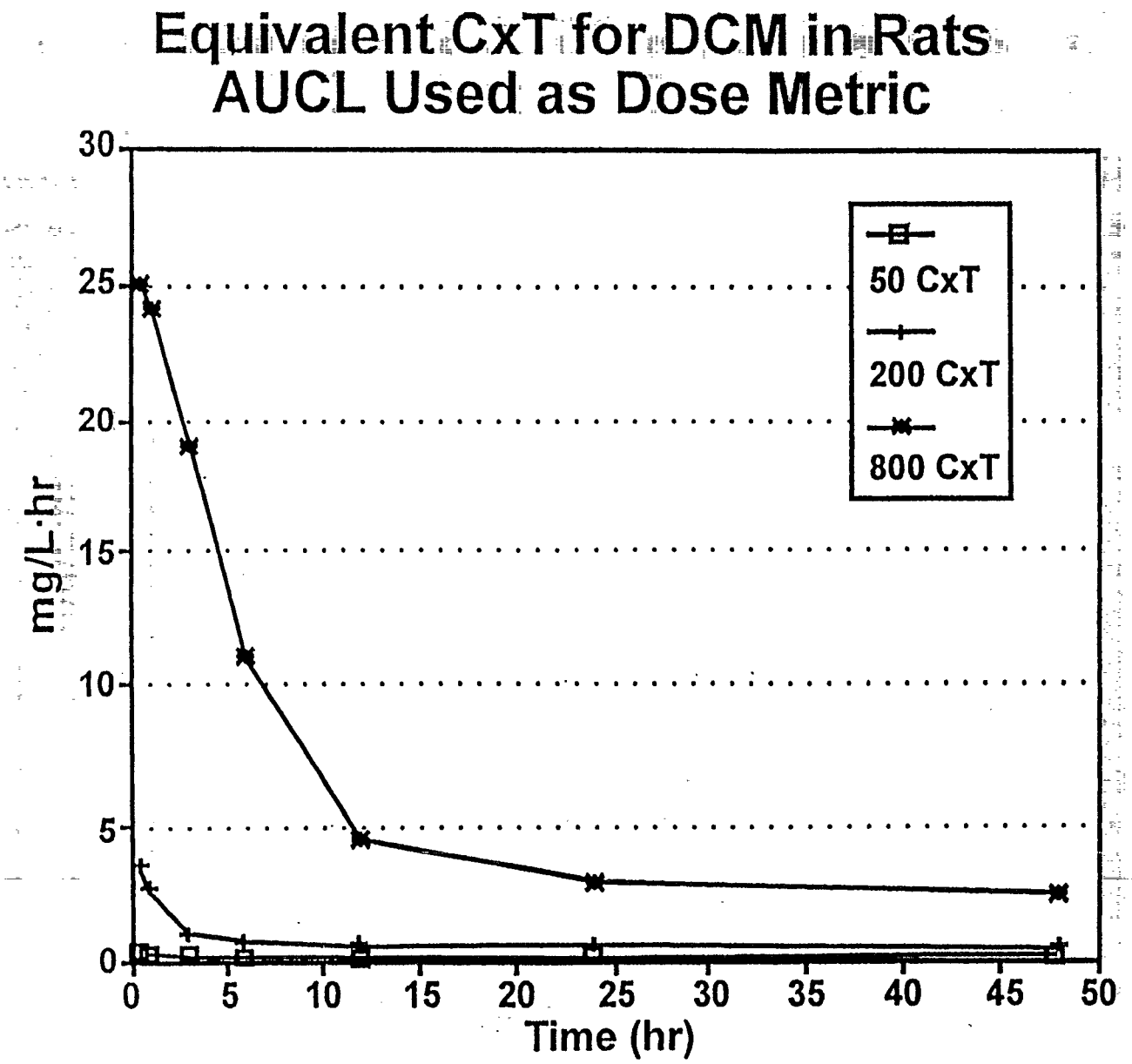
- Equivalent effects will be observed at equal tissue exposure/dose when measured by the appropriate metric.

- Dosimetry provides an adequate basis for identifying pharmacodynamic differences.

Reasons for understanding dosimetry in looking at concentration-time relationships include:

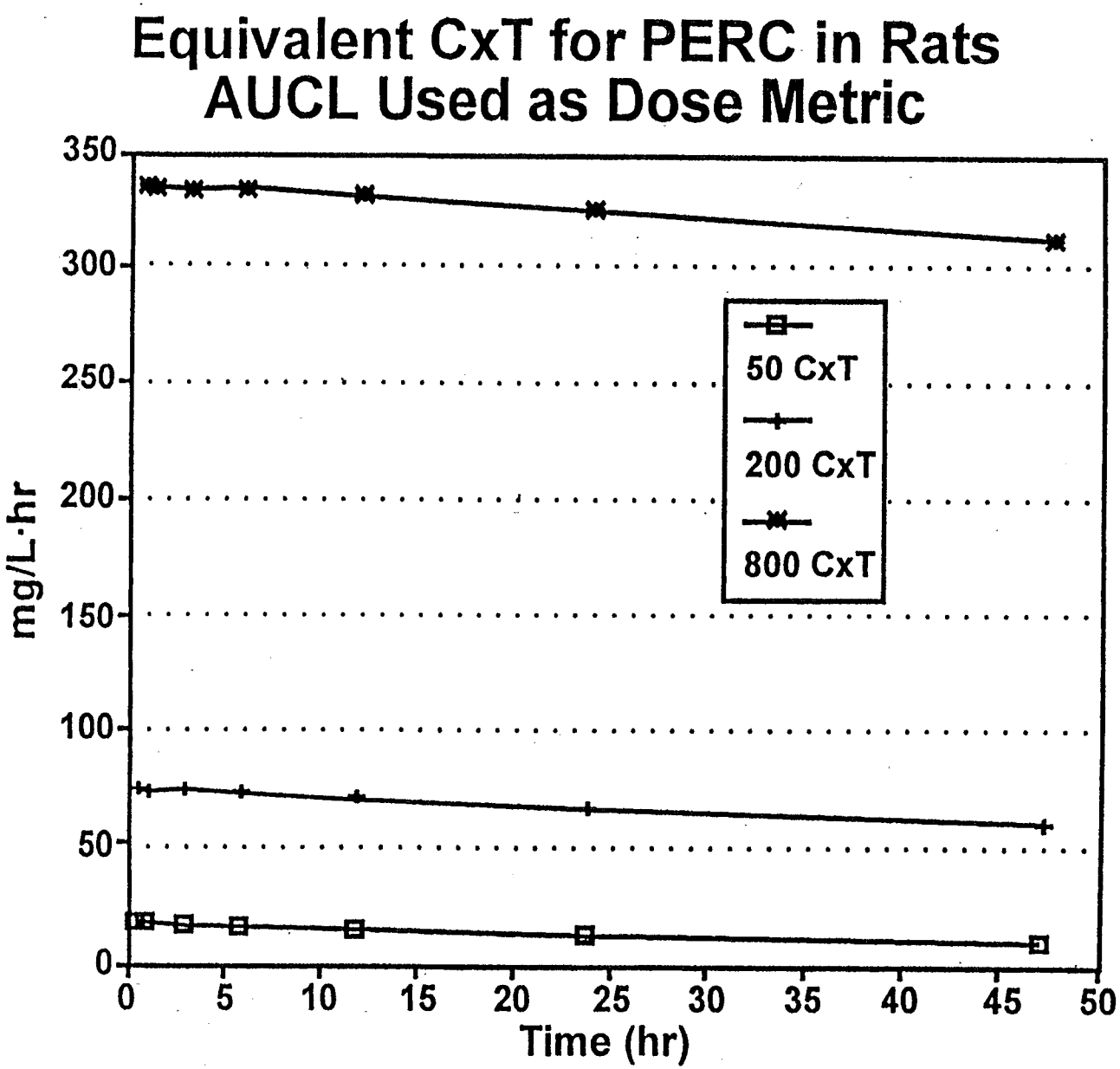
- Nonlinear uptake, metabolism, or clearance
- Toxicity associated with products of metabolism rather than the parent chemical
- Tissue interactions (depletion of critical resources, induction of clearance/repair)

To demonstrate the importance of understanding dosimetry when trying to interpret concentration-time relationships, the following example shows the impact of saturable metabolism on the apparent concentration-time (C × T) relationship for the production of carbon monoxide (CO) from two dihalomethanes: dichloromethane (DCM) and bromochloromethane (BCM). The PBPK model used in this



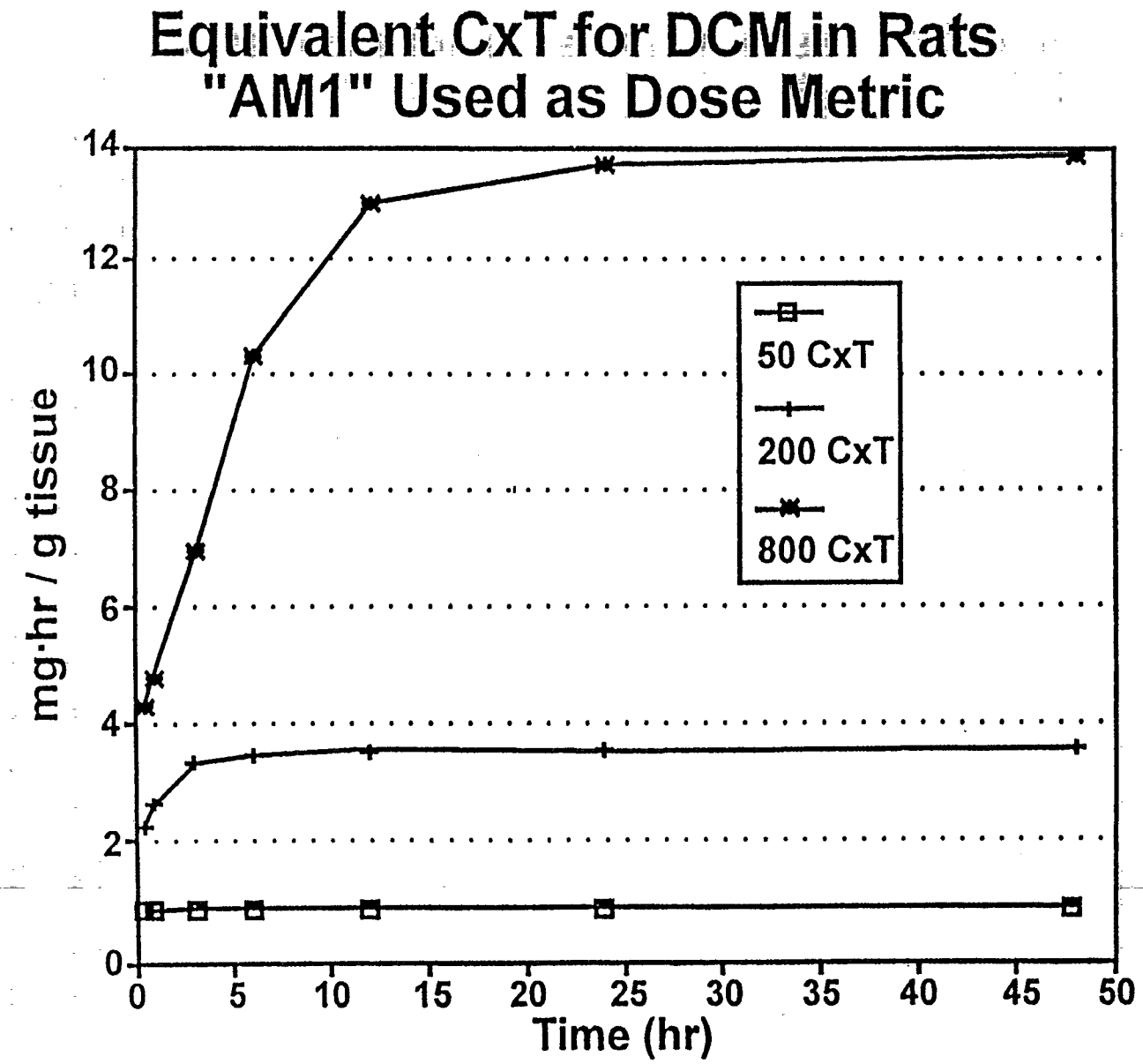
(Jarabek and McDougal, 1993)

Figure 5-12



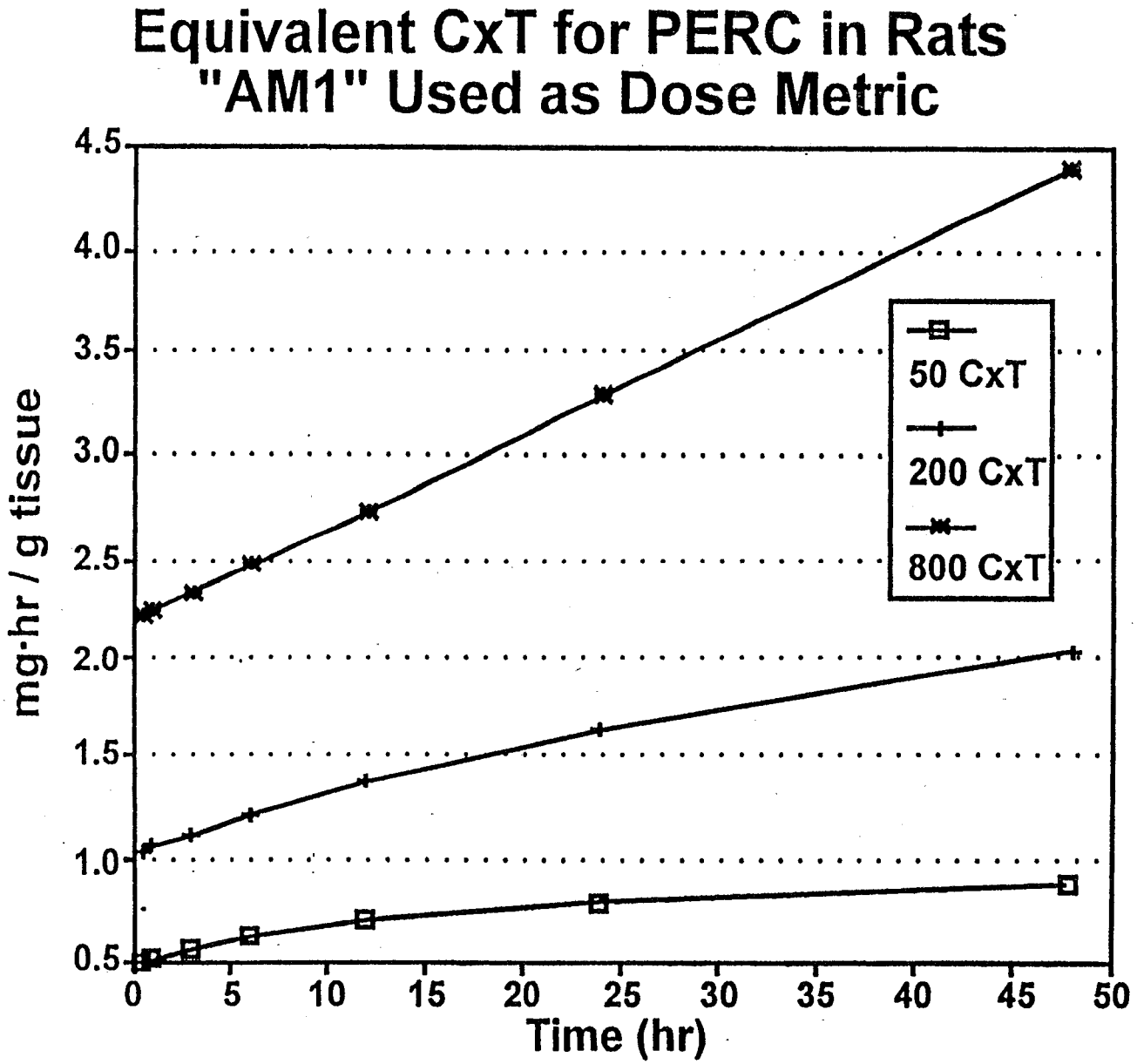
(Jarabek and McDougal, 1993)

Figure 5-13



(Jarabek and McDougal, 1993)

Figure 5-14



(Jarabek and McDougal, 1993)

Figure 5-15

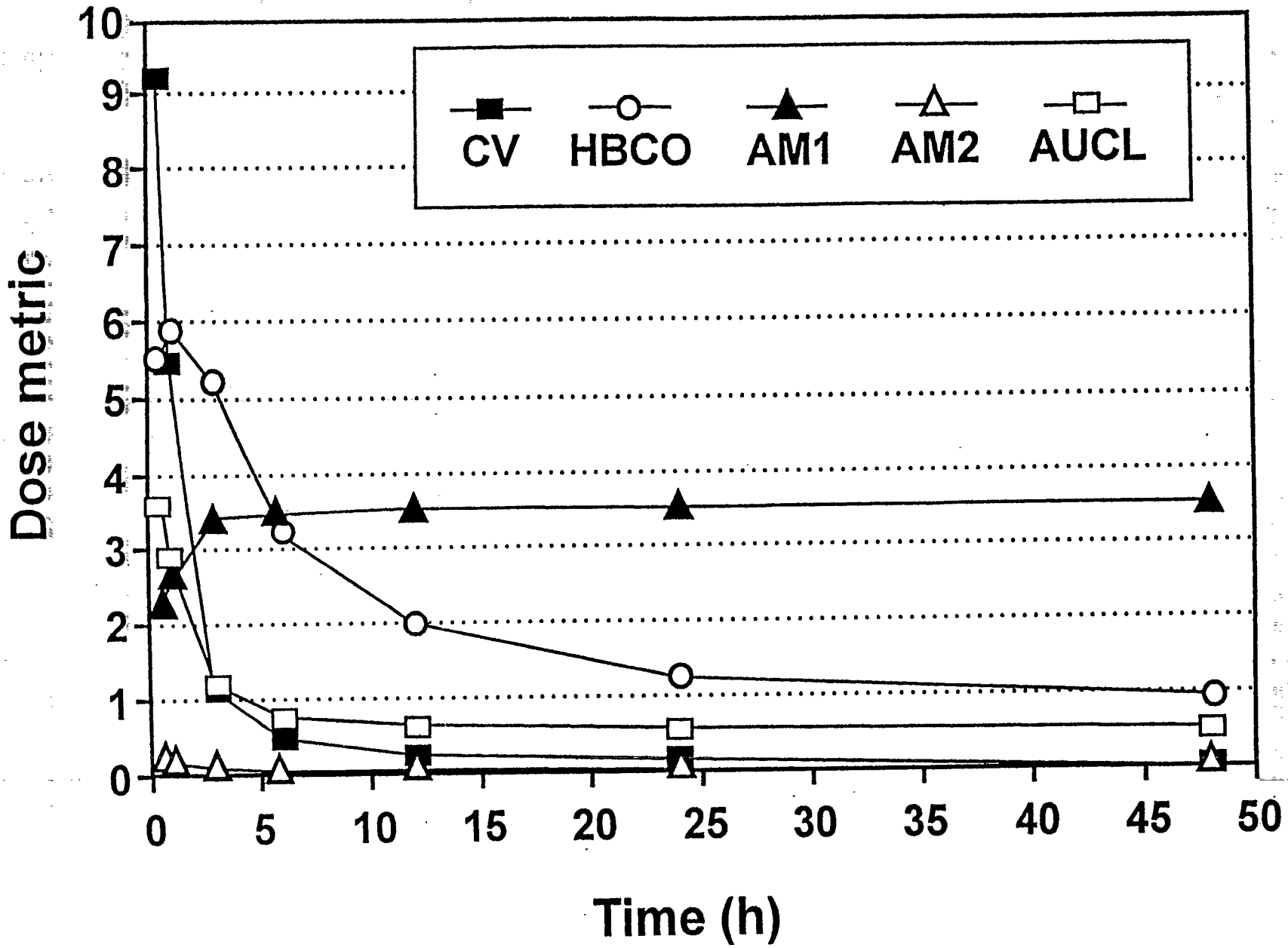

CV
HBCO
AM1
AM2
AUCL
Dose metric
Time (h)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
40
45
50

Figure 5-16

example[1] not only tracks the parent chemical but also the CO produced by metabolism. Two metabolic pathways are described: a saturable oxidative (P450) pathway which produces both carbon dioxide (CO_2) and CO, and a first-order glutathione conjugation pathway which produces only CO_2. The metabolite model includes a fairly complete description of the fate of the CO produced and is able to predict the fraction of hemoglobin bound up as carboxyhemoglobin at any time, as determined by the current rate of CO production (and inhalation of CO, if appropriate), the competition of oxygen and CO for hemoglobin, and the rate of exhalation of unbound CO. This PBPK model has also been successfully applied to a variety of exposure routes: inhalation, intravenous, and oral.

The ability of the PBPK model to describe the kinetics of CO exposure is shown in Figure 5-17, which shows the model predictions (lines) and experimental data (points) for blood carboxyhemoglobin (HbCO) levels from human volunteers exposed to CO at various concentrations. Figure 5-18 shows the concentration-time profile for CO exposures associated with an HbCO level of 40%, as predicted by the model (solid line). The profile for C × T=800 ppm-hours is also shown (dotted line) for comparison. It can be seen that extrapolation on the basis of C × T is reasonably accurate, although it tends to be overconservative at long durations and under conservative at short durations.

The situation is quite different, however, for the production of CO from dihalomethanes. Figure 5-19 shows the PBPK model predictions (lines) and data (points) for short duration (4-hr), high concentration exposures of rats to DCM. The shaded area indicates the exposure period. Due to saturation of metabolism, approximately the same blood HbCO levels are achieved at concentrations of 200 and 1014 ppm; however, due to post-exposure metabolism of the unmetabolized DCM stored in the fat, this HbCO level was maintained for a period of time after the exposure was terminated, particularly at the higher concentration. Thus the C × T relationship for CO toxicity from DCM exposure is greatly distorted by the impact of nonlinear metabolism.

Similarly, Figure 5-20 shows the predicted (lines) and experimental timecourse for blood HbCO levels in rats following a very short (1/2 hr), very high concentration (5000 ppm) exposure to BCM (triangles) or DCM (circles). Again, the shaded area indicates the exposure period. The higher peak HbCO level for BCM reflects the higher capacity of P450 metabolism for this compound, while the longer post-exposure metabolism of BCM reflects its greater tissue solubility (resulting in greater storage). Thus the extent of the impact of nonlinear metabolism on the apparent C × T relationship is chemical dependent.

Figure 5-21 shows that for sufficiently high concentrations of another compound, halothane, the amount of metabolism which takes place after the cessation of a 4-hr exposure (as measured by the production of bromide) can actually equal the amount that takes place during the exposure. Figures 5-22 and 5-23 show PBPK model simulations of the same scenario for two different compounds, vinyl chloride (VC) and carbon tetrachloride (CCl_4). Note that in the case of VC (Figure 5-22), the low tissue solubility of the chemical prevents significant post-exposure metabolism, so that the amount metabolized 24 hours after the beginning of a 6-hr exposure is not much greater than the amount metabolized immediately after the 6-hr exposure. At high concentrations of CCl_4, however, the amount metabolized in one day for a 6-hr exposure is almost as much as the amount metabolized for a continuous exposure.

---

[1]Andersen ME, Clewell HJ, Gargas ML, MacNaughton MG, Reitz RH, Nolan RJ, McKenna MJ. 1991. Physiologically based pharmacokinetics modeling with dichloromethane, its metabolite carbon monoxide, and blood carboxyhemoglobin in rats and humans. *Toxicol Appl Pharmacol* 108:14-27.

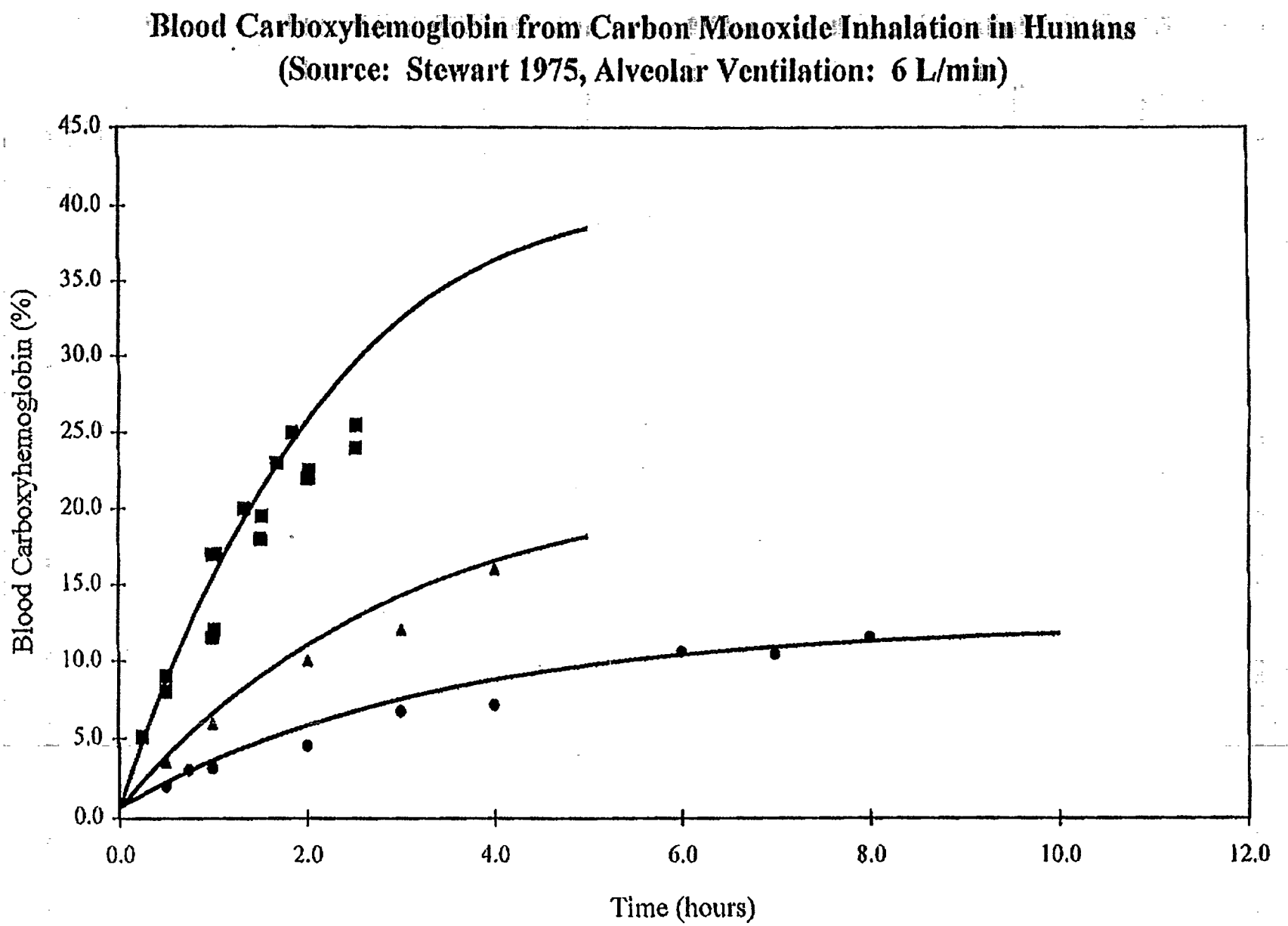

Blood Carboxyhemoglobin from Carbon Monoxide Inhalation in Humans
(Source: Stewart 1975, Alveolar Ventilation: 6 L/min)
Blood Carboxyhemoglobin (%)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Time (hours)

Figure 5-17

Blood Carboxyhemoglobin from Carbon Monoxide Inhalation for Different Durations Assuming Alveolar Ventilation for Light Work (16 L/min)

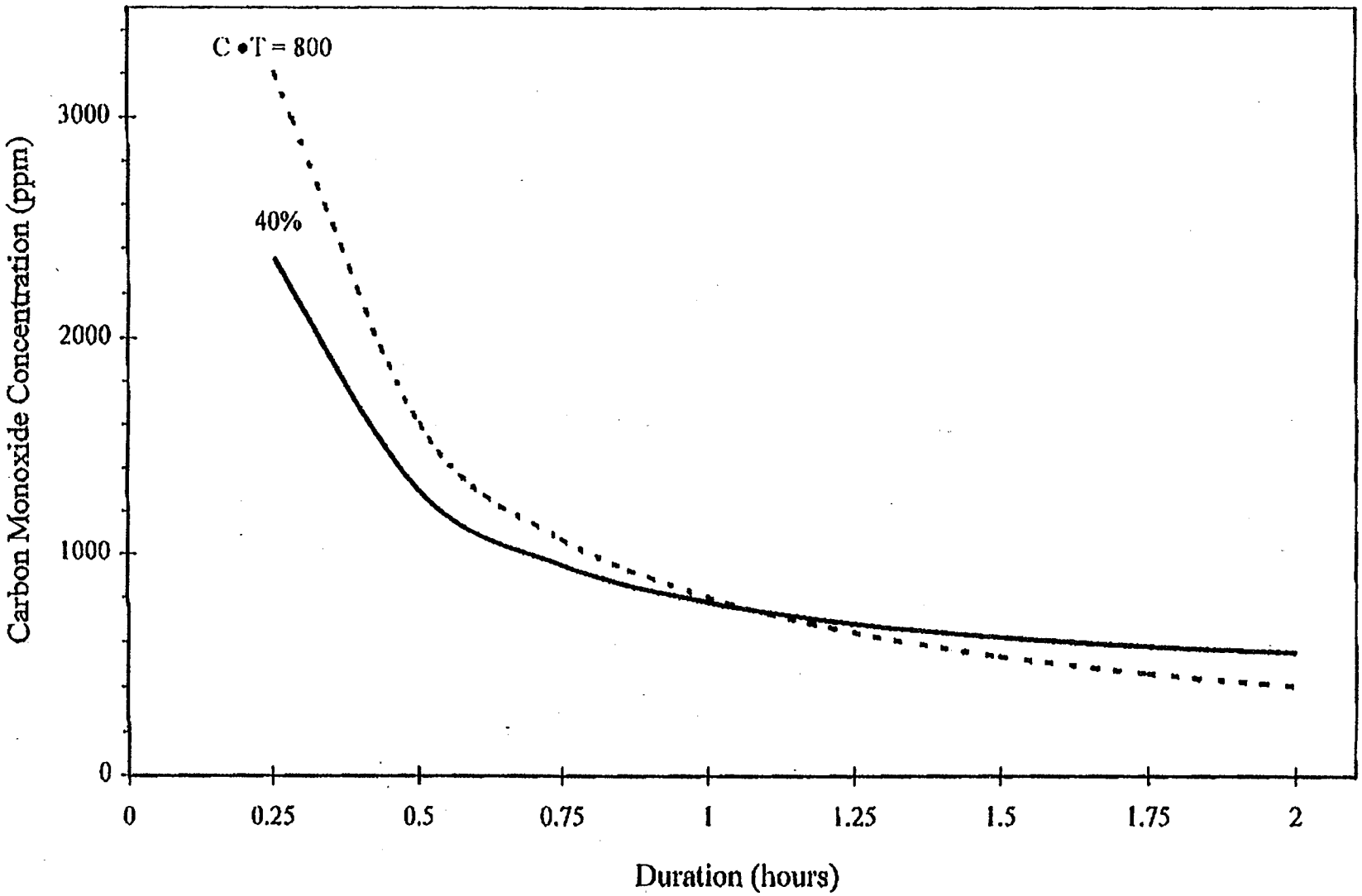


Figure 5-18

Figure 5-20

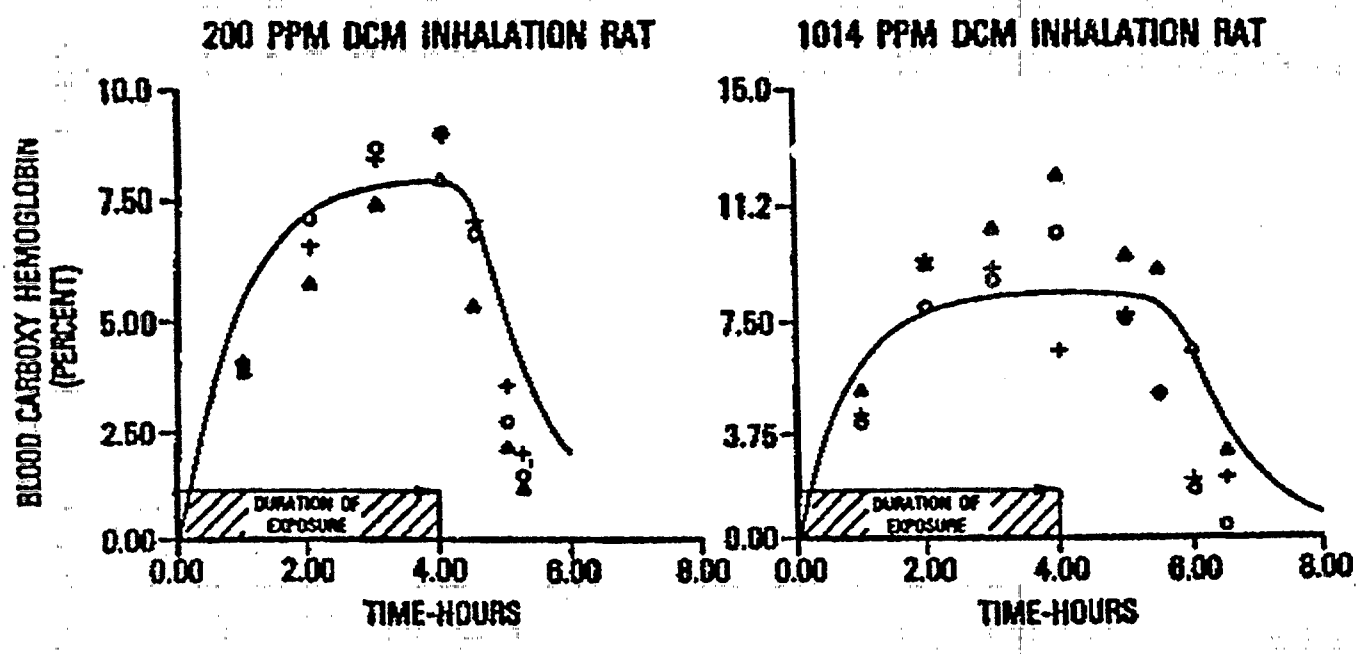

200 PPM DCM INHALATION RAT
1014 PPM DCM INHALATION RAT
BLOOD CARBOXY HEMOGLOBIN (PERCENT)
10.0
7.50
5.00
2.50
0.00
15.0
11.2
7.50
3.75
0.00
DURATION OF EXPOSURE
0.00
2.00
4.00
6.00
8.00
TIME-HOURS

Figure 5-19

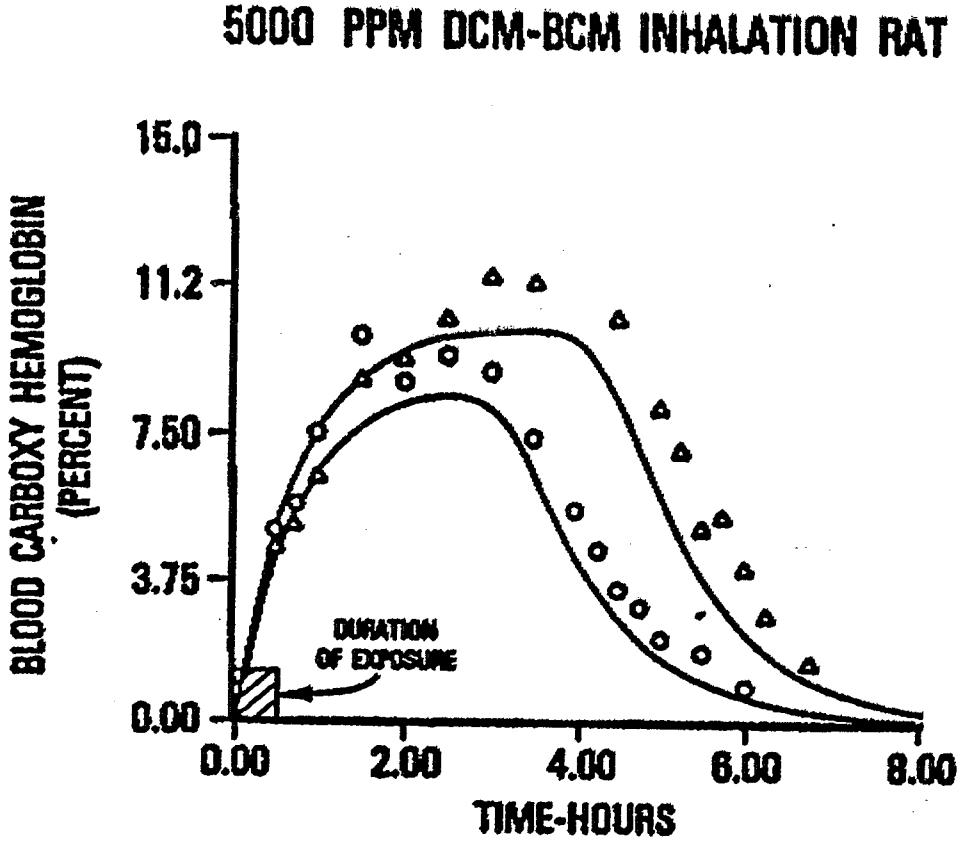

5000 PPM DCM-BCM INHALATION RAT
BLOOD CARBOXY HEMOGLOBIN (PERCENT)
15.0
11.2
7.50
3.75
0.00
DURATION OF EXPOSURE
0.00
2.00
4.00
6.00
8.00
TIME-HOURS

Figure 5-20

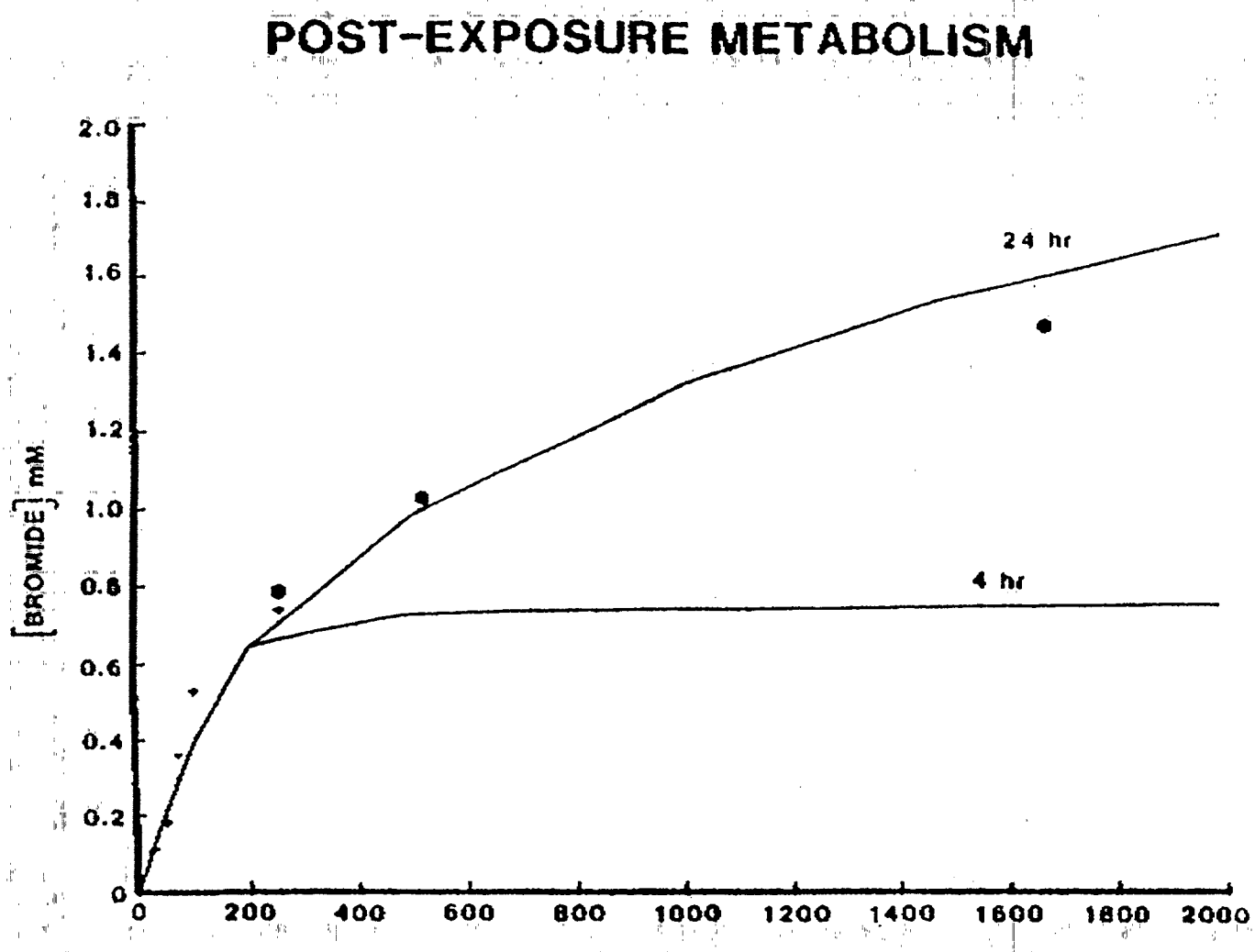

POST-EXPOSURE METABOLISM
[BROMIDE] mM
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400
1600
1800
2000
24 hr
4 hr

Figure 5-21

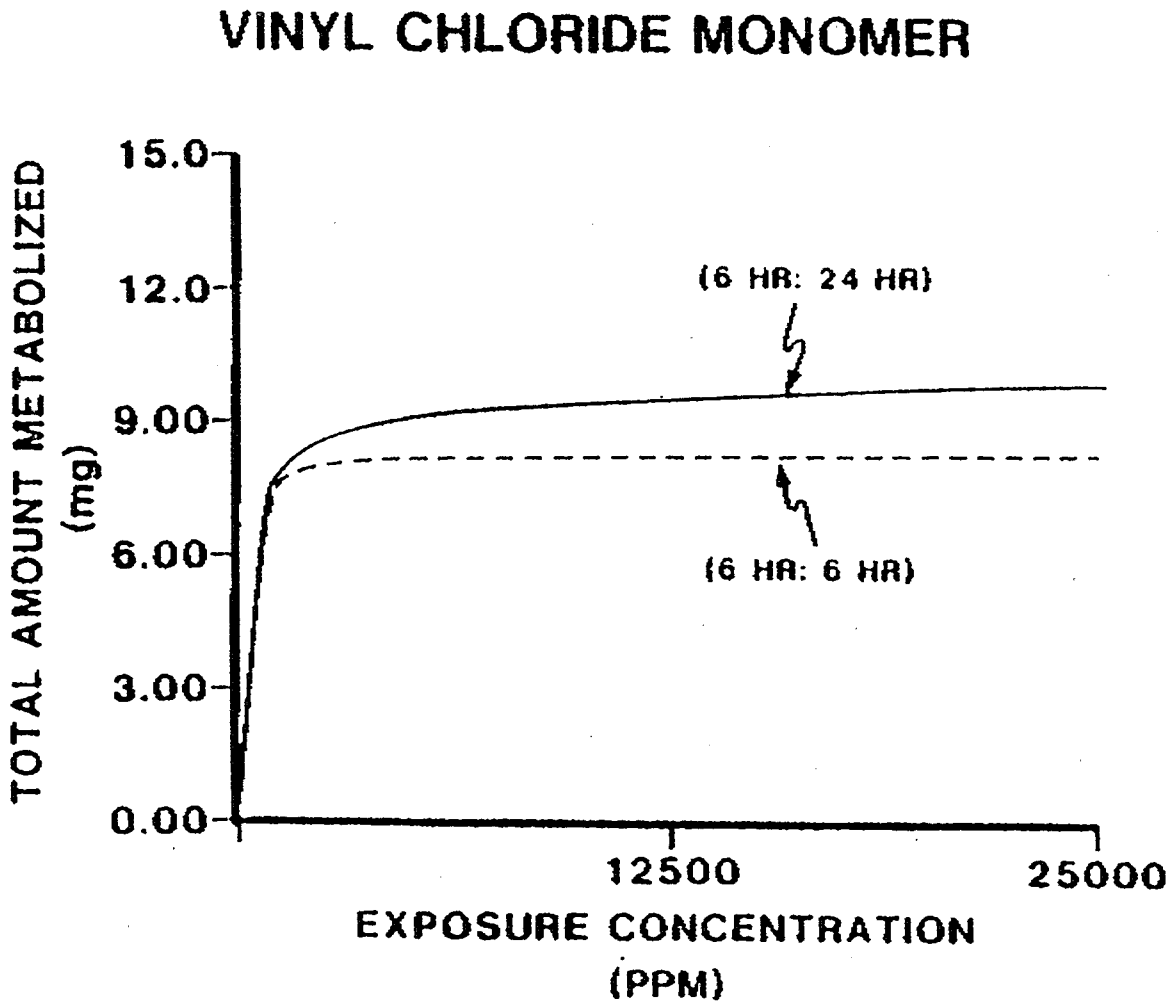

VINYL CHLORIDE MONOMER
TOTAL AMOUNT METABOLIZED (mg)
15.0
12.0
9.00
6.00
3.00
0.00
(6 HR: 24 HR)
(6 HR: 6 HR)
12500
25000
EXPOSURE CONCENTRATION (PPM)

Figure 5-22

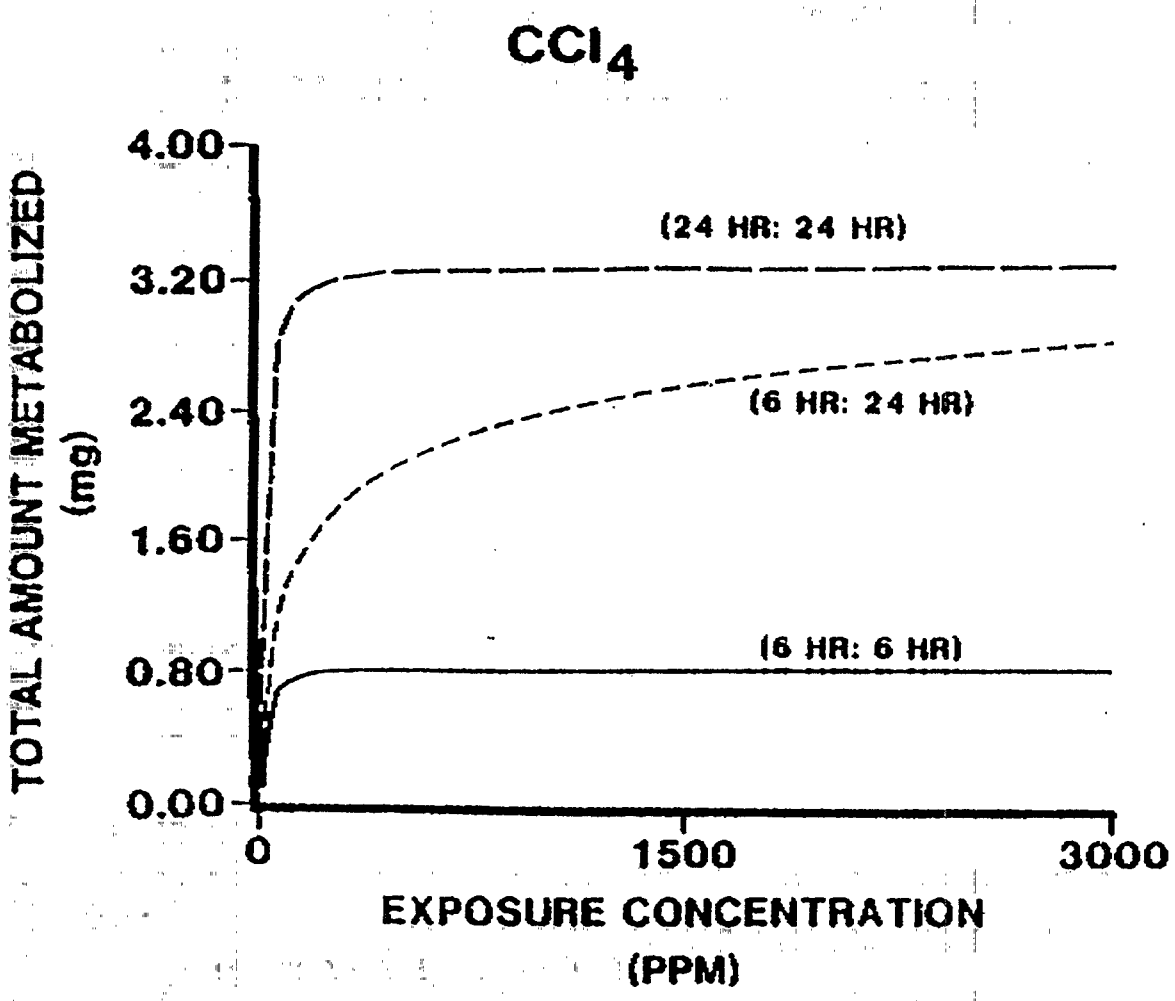

CCl4
TOTAL AMOUNT METABOLIZED (mg)
4.00
3.20
2.40
1.60
0.80
0.00
(24 HR: 24 HR)
(6 HR: 24 HR)
(6 HR: 6 HR)
0
1500
3000
EXPOSURE CONCENTRATION (PPM)

Figure 5-23

Finally, it should be noted that the impact of post-exposure metabolism will, in general, be different for human exposures as compared to similar rodent exposures. Figure 5-24 shows the PBPK simulation for the case of CCl_4; the longer time-constants for chemical kinetics in humans lead to a very different timecourse for the chemical compared to the rat.

The appropriate dose metrics will vary depending on the toxicity and the chemical:

- Stable chemicals: area under the curve ($C \times T$)
- Acute toxicity: peak concentration or $C^n \times T$
- Reactive intermediates: amount produced/tissue volume
- U.S. EPA RfD guidelines: mg/kg body weight
- U.S. EPA RfC guidelines (Category 3 gas): duration-adjusted exposure concentration (x ratio of partition coefficients if >1)

Mode of action is important in choosing an appropriate measure of tissue dose:

- Parent chemical vs. stable metabolite vs. reactive intermediate
- Reactivity vs. physical effect vs. receptor occupancy
- Cumulative vs. rapidly reversible

As depicted in Figure 5-25, for the case of trichloroethylene (TCE), multiple dose metrics may be required for different toxicities associated with the same chemical. For example, in the case of TCE, acute toxicity might be produced by the parent chemical and the best metric might be either the maximum concentration (C_{max}) or area under the concentration curve (AUC) for TCE. However, other toxicities of TCE might be the result of the activity of one of the stable metabolites of TCE, such as dichloroacetic acid (DCA), trichloroacetic acid (TCA) or trichloroethanol (TCOH). The best metric in such cases might be a measure of exposure of the target tissue to that metabolite, whether the Cmax, AUC, or time above a critical concentration (TACC). Finally, some toxicities might result from reactive species produced during metabolism, in which case an appropriate measure might be the rate of production of the reactive species divided by the volume of the tissue into which they are produced.

Dosimetry for developmental toxicity is even more complicated due to the need to consider the relationship of the exposure period to the time course of pregnancy and gestation. The importance of this relationship can be demonstrated in the case of methylmercury (MeHg). Figure 5-26 shows the ability of our PBPK model of MeHg to simulate the time course for maternal and fetal MeHg levels associated with an acute exposure of the mother to MeHg from contaminated grain.[2] Figure 5-27 shows simulations performed with this model to evaluate the impact of the timing of the exposure with respect to the pregnancy. As can be seen in this figure, the third-trimester fetal exposure to MeHg resulting from the same maternal exposure can vary by as much as a factor of 3 depending on the relationship between the exposure and gestation.

---

[2]Gearhart JM, Clewell HJ, Crump KS, Shipp AM, Silvers, A. 1995. Pharmacokinetics dose estimates of mercury in children and dose-response curves of performance tests in a large epidemiological study. *Water Air Soil Pollut* 80:49-58.

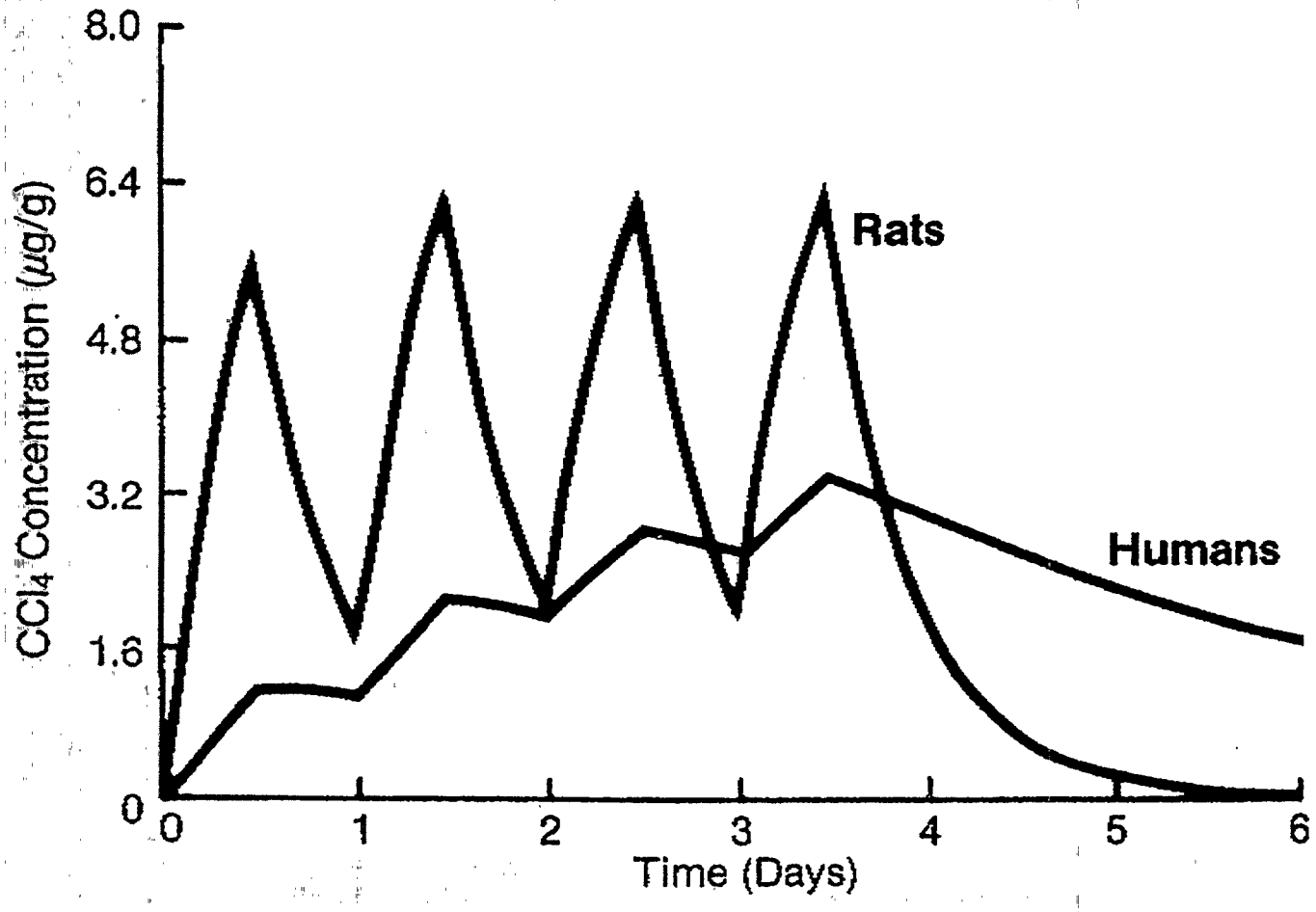

8.0
6.4
4.8
3.2
1.6
0
CCl_4 Concentration (µg/g)
Rats
Humans
0
1
2
3
4
5
6
Time (Days)

Figure 5-24

Multiple Dose Metrics: Trichloroethylene

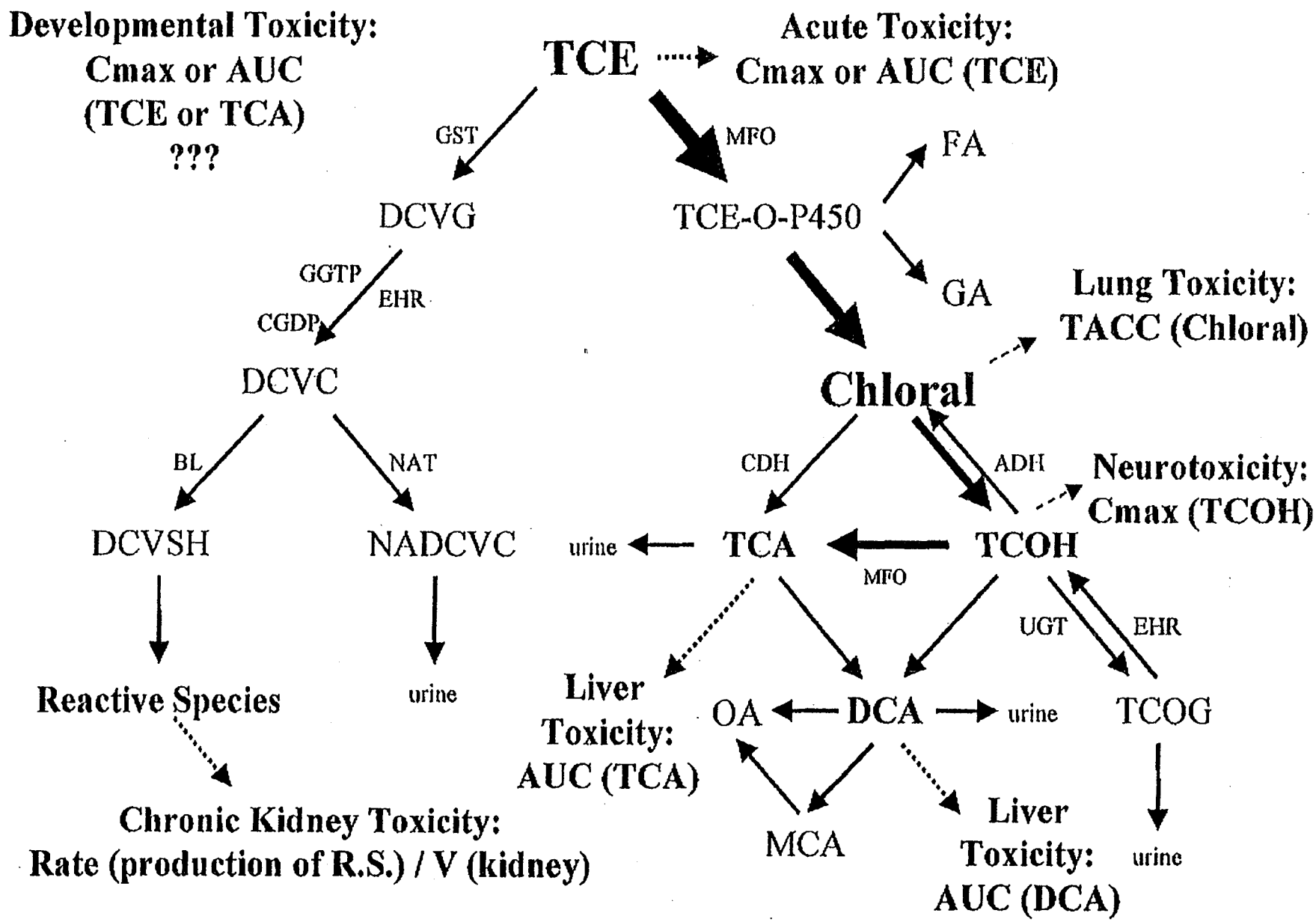


Figure 5-25

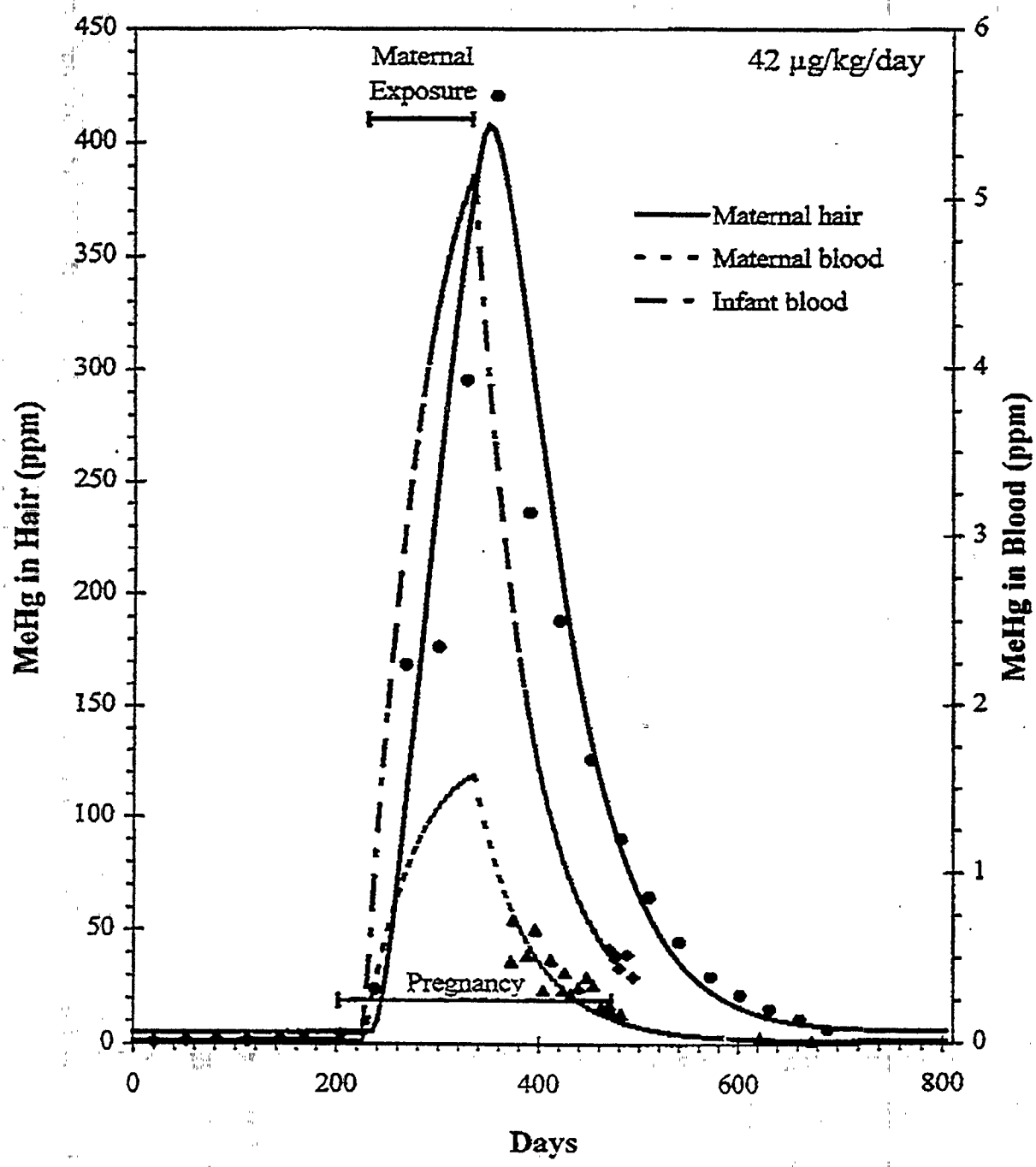
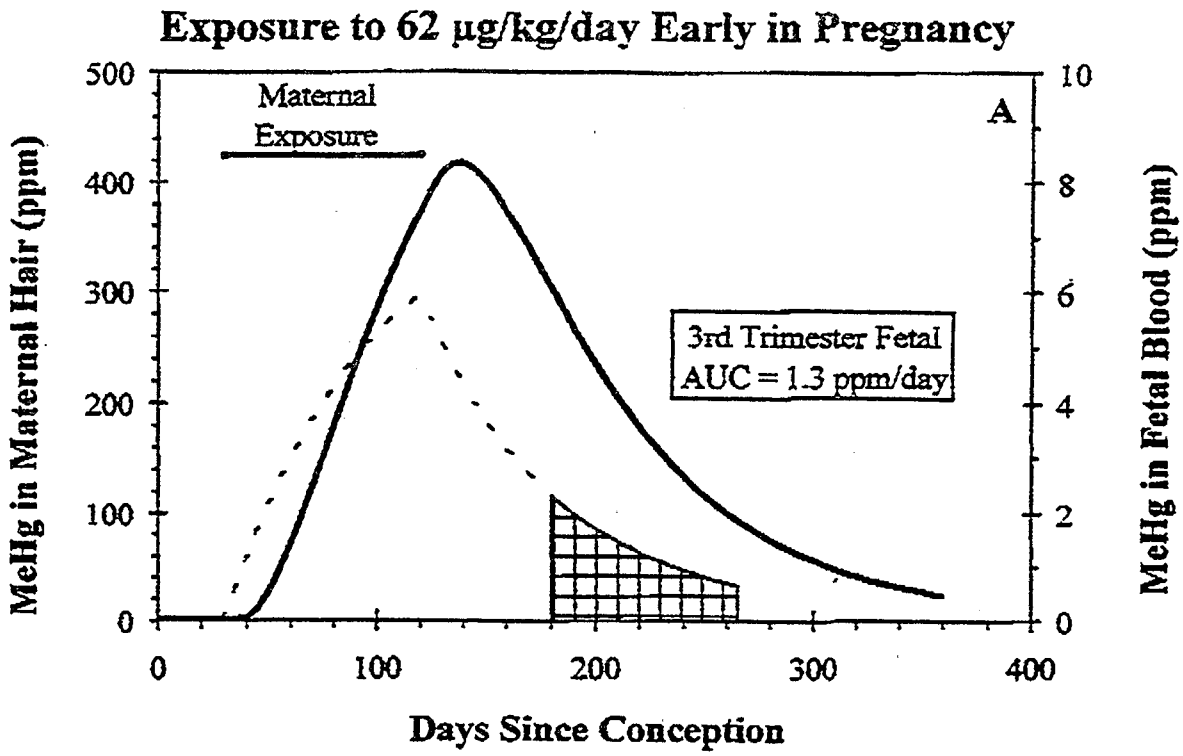

Maternal
Exposure
42 µg/kg/day
Maternal hair
Maternal blood
Infant blood
Pregnancy
MeHg in Hair (ppm)
MeHg in Blood (ppm)
Days
0
50
100
150
200
250
300
350
400
450
0
1
2
3
4
5
6
0
200
400
600
800

Figure 5-26


Exposure to 62 µg/kg/day Early in Pregnancy
Maternal Exposure
A
3rd Trimester Fetal AUC = 1.3 ppm/day
MeHg in Maternal Hair (ppm)
MeHg in Fetal Blood (ppm)
Days Since Conception
0
100
200
300
400
500
0
2
4
6
8
10

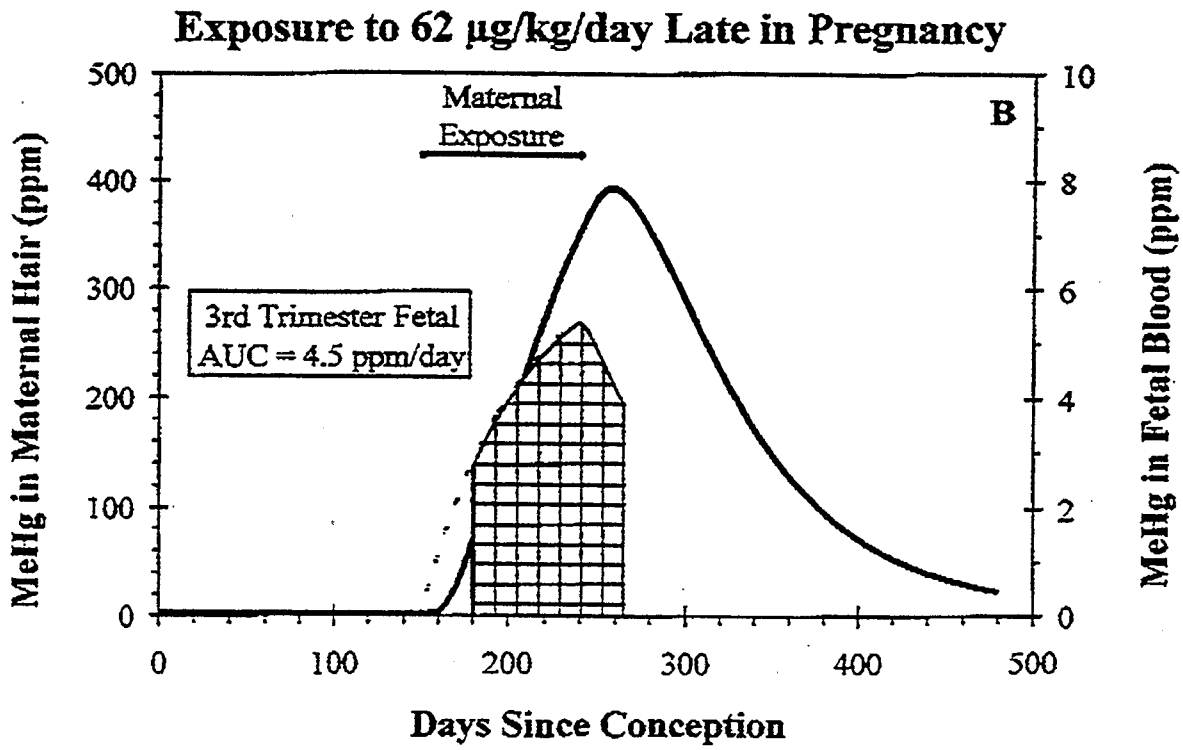

Exposure to 62 µg/kg/day Late in Pregnancy
Maternal Exposure
B
3rd Trimester Fetal AUC = 4.5 ppm/day
MeHg in Maternal Hair (ppm)
MeHg in Fetal Blood (ppm)
Days Since Conception
0
100
200
300
400
500
0
2
4
6
8
10

Figure 5-27

To demonstrate the importance of selecting an appropriate dose measure based on the mode of action for the toxicity of concern, PBPK models for TCE and VC were exercised to determine general expectations for the cross-species dosimetry for one class of chemicals, the volatile lipophilic solvents.[3] All three of these chemicals would be considered Category 3 gases (relatively water-insoluble chemicals which achieve a steady-state during inhalation exposure) in the EPA (1994) dosimetry guidelines. In a standard risk assessment, the animal-to-human dosimetry adjustment for each of these chemicals would be performed in exactly the same way, based on time-weighted average (TWA) exposure concentration for inhalation and on mg/kg/day administered dose for oral exposure, regardless of the nature of the toxic endpoint or the mechanism of toxicity. As shown below, the correct relationship for cross-species dosimetry depends on whether the toxicity is due to the parent chemical or a metabolite, and in the case of toxicity from a metabolite, whether the metabolite is reactive or stable. Moreover, the nature of the cross-species relationship for each of these possibilities is different for oral exposure than for inhalation. Therefore, pharmacokinetics modeling is required to provide accurate cross-species extrapolation that considers the nature of the toxic entity.

For inhalation exposure:

- Acute toxicity due to parent chemical (TCE, VC)
  —PBPK HEC *similar* to default

- Chronic toxicity due to reactive metabolite (VC)
  —PBPK HEC 5- to 25-fold *higher* than default

- Chronic toxicity due to stable metabolite (TCE)
  —PBPK *similar* to 10-fold *lower* than default

For oral exposure:

- Acute toxicity due to parent chemical (TCE, VC)
  —PBPK human dose 10- to 100-fold *lower* than RfD default

- Chronic toxicity due to reactive metabolite (VC)
  —PBPK human dose *similar* to RfD default

- Chronic toxicity due to stable metabolite (TCE)
  —PBPK human dose 15- to 60-fold *lower* than RfD default

Conclusions regarding use of mechanistic modeling in dosimetry include:

- Advantage: Provides broader selection of potential dose metrics (including stable and reactive metabolites).

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[3]Clewell HJ III, Gentry PR, Gearhart JM. 1997. Investigation of the potential impact of benchmark dose and pharmacokinetics modeling in noncancer risk assessment. *J Toxicol Environ Health* 52:475-515.

- Greatest impact: Accounts for nonlinearities in uptake, metabolism, and clearance that alter the C × T relationship.

- Challenge: Selection of dose metric *for each toxic endpoint* is based on presumed mechanism of action and available experimental data (whether using default *or* mechanistic dosimetry).

- Caution: When incorporating dosimetry, it is important to considering pharmacodynamics (progression, compensation, repair) to produce the appropriate duration adjustment.

SECTION SIX

PLENARY PRESENTATIONS: IMPLICATIONS FOR RISK ASSESSMENT

6.1 IMPLICATIONS FOR RISK ASSESSMENT

Steve Rappaport, University of North Carolina

Risk assessment represents the quantification of the exposure-risk relationship. It can be done in a variety of ways (epidemiology, toxicology with extrapolation, deterministic models, guessing). We should use human data for risk assessment whenever possible. Poor human data is arguably more valuable than really good animal data for risk assessment.

Exposures in human beings vary widely. Figure 6-1 presents data from a longitudinal study over 1 year of occupational exposure to styrene. Levels varied 100- to 1,000-fold between workers and within workers over time.

The C × T debate relates to the time series of exposure and tissue levels. Do peaks of exposure influence the rate at which the chemical is absorbed into the body, metabolized, etc.? Haber's law relates to the saturable processes. At low levels of exposure, these tend to be linear and Haber's law is approximately true; Haber's law is never true at high levels.

We can also distinguish between acute and chronic effects. Doing risk assessment for acute effects is problematic. The episodes that generate very high levels (excluding irritation as an endpoint) are likely to result from unusual accidents in occupational settings or catastrophes in ambient settings, and it is difficult to model the behavior in such events. In these situations, risk assessment becomes surreal because nonlinear models are unstable since the output depends largely on initial conditions. Therefore, the focus should be on preventing the accidents and catastrophes (the source rather than the receptor).

For chronic effects, the C × T debate becomes more interesting. We can investigate it with a combination of mechanistic and kinetic models *plus* biomarkers. Interindividual variability becomes important, because the rates of uptake, bioactivation, detoxification, repair, etc. tend to be specific to particular individuals. Figure 6-2 presents a simple model relating exposure to tissue burden. Figure 6-3 shows this relationship in a hypothetical example of a realistic exposure scenario. Substances producing chronic effects include slowly eliminated substances, such as lead (Figures 6-4 and 6-5) and rapidly eliminated substances such as styrene (Figures 6-6 and 6-7). In both of these examples, evidence of linearity between tissue levels and external exposure is evident.

In summary, we should focus the Haber's law debate on substances that produce chronic effects. We also should differentiate between substances that are slowly eliminated (for which the C × T debate only exists only over very long time periods) and those that are rapidly eliminated (for which the C × T debate becomes more interesting, and for which we should use human data and biomarkers to get at the linearity of these processes).

Exposure varies over time and between persons

- **Example: Workers exposed to styrene in a boat plant**

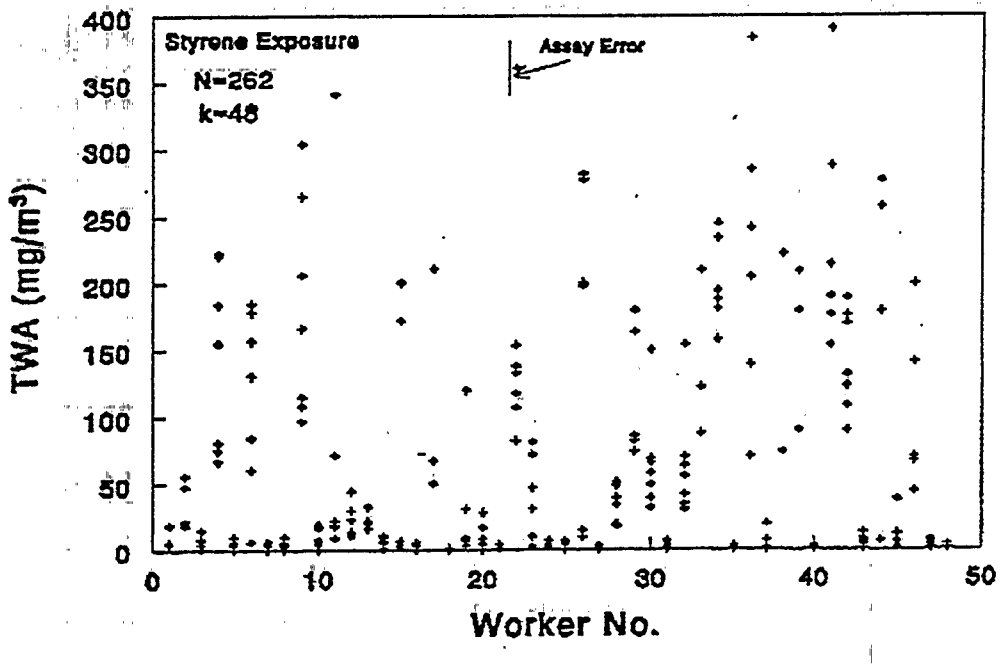


Figure 6-1

RISKS OF CHRONIC DISEASE

If Haber's Law holds: Dose and risk are proportional to cumulative exposure

- **For one person exposed to time series $\{X_t\}$:**

$$\{X_t\} \xrightarrow[\text{(Zero Order)}]{\text{Uptake}\ k_o} \{P_t\} \xrightarrow[\text{(First Order)}]{\text{Elimination, metabolism, etc.}\ k_1} \text{Subsequent time series}$$

EXPOSURE BURDEN

$$P_t = \frac{k_0}{k_1} X_t (1 - e^{-k_1 \Delta t}) + P_{t-1} e^{-k_1 \Delta t}$$

Increment from current exposure + Residual burden from prior exposures

- **Cumulative Exposure (CE):**

$$CE = \mu_X \cdot t$$

$$DOSE_P = \sum_{j=1}^{n} P_j \Delta t = \mu_P \cdot t = \frac{k_0}{k_1} \cdot CE$$ where μ_P is the mean burden over time

- **Note that for a population exposed to $\{X_t\}$, k_o and k_1 are random variables!**

Figure 6-2

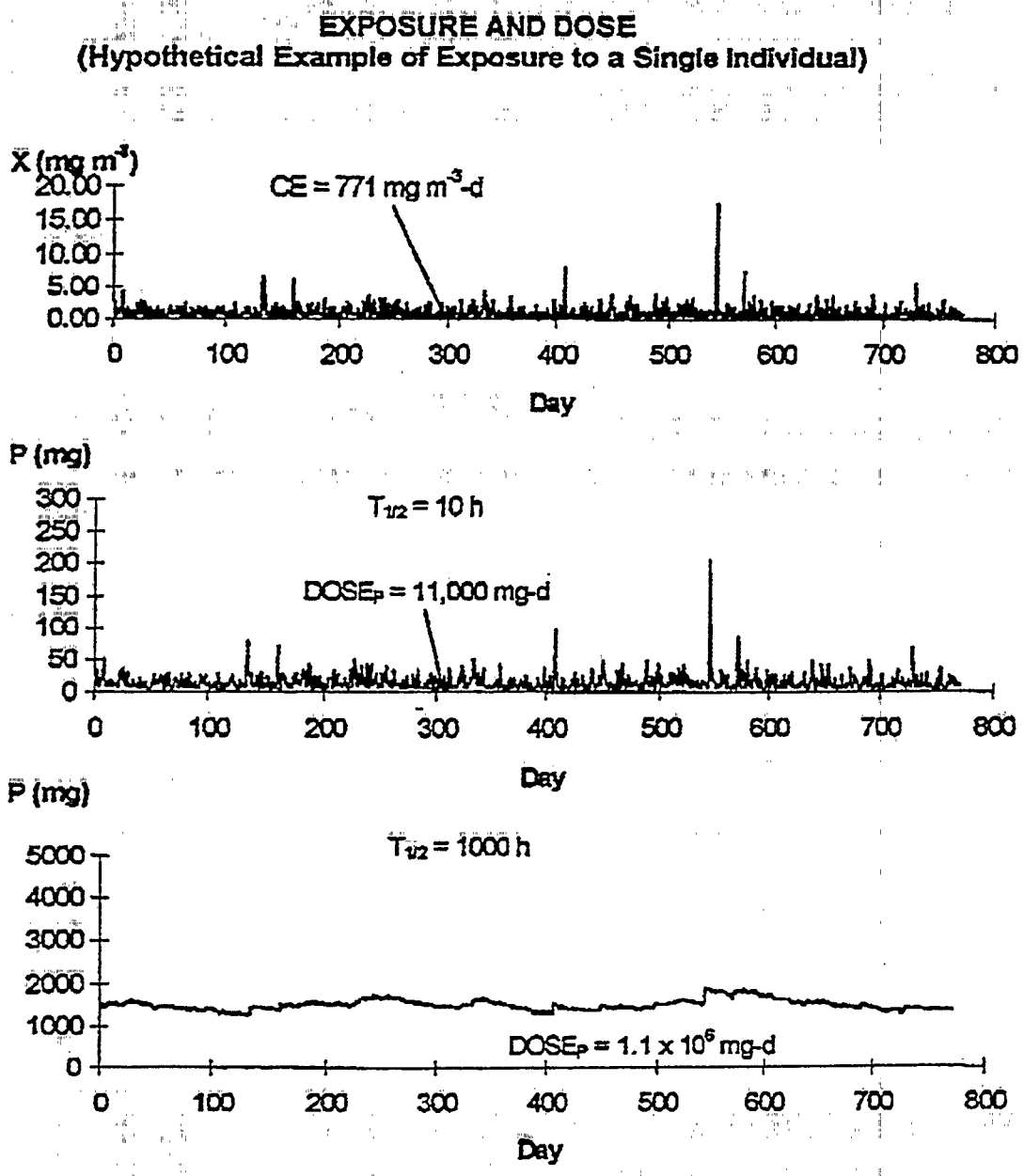

EXPOSURE AND DOSE
(Hypothetical Example of Exposure to a Single Individual)
X (mg m-3)
CE = 771 mg m-3-d
20.00
15.00
10.00
5.00
0.00
0
100
200
300
400
500
600
700
800
Day
P (mg)
T1/2 = 10 h
DOSEP = 11,000 mg-d
300
250
200
150
100
50
0
Day
P (mg)
T1/2 = 1000 h
5000
4000
3000
2000
1000
0
DOSEP = 1.1 x 10^6 mg-d
Day

Figure 6-3

Exposure To Inorganic Lead Among Alkyl-Lead Workers

Ref.: Rappaport, SM . *Ann. Occup. Hyg.* 35, 61-121, 1991), data from Cope et al. *AIHAJ* 40, 372-379, 1979.

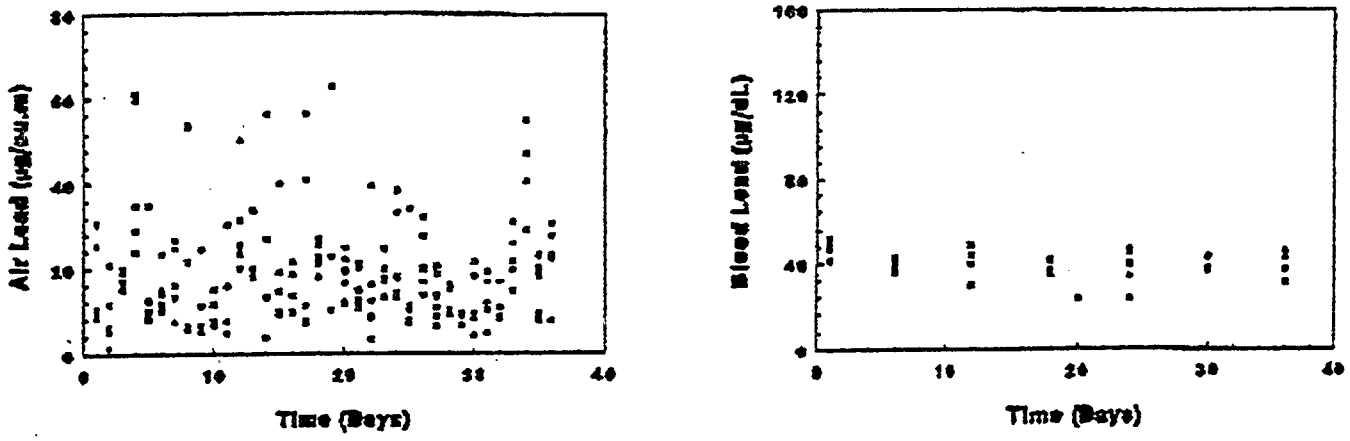


Figure 6-4

UPTAKE OF LEAD AMONG BATTERY WORKERS

Ref: Rappaport SM (1995) *Toxicol. Letters* 77: 171-182, data from Hodgkins DG, et al. (1992) *Br. J. Ind. Med.* 49:241-248.

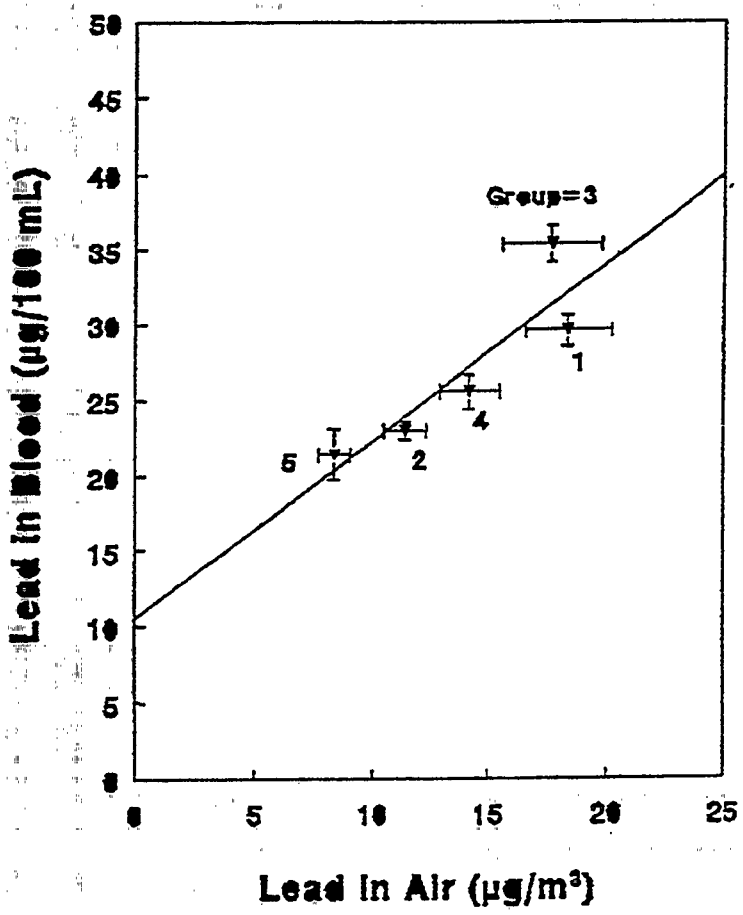
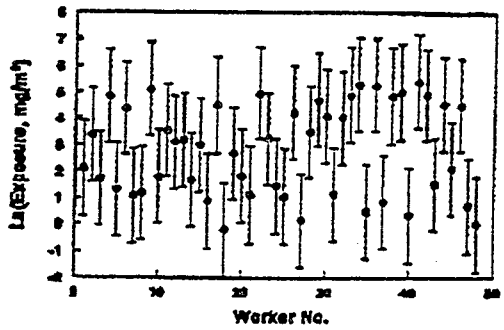


Figure 6-5

STYRENE-EXPOSED BOAT WORKERS
(Est. mean values and SEs shown for logged data)

Exposure



Styrene in Breath

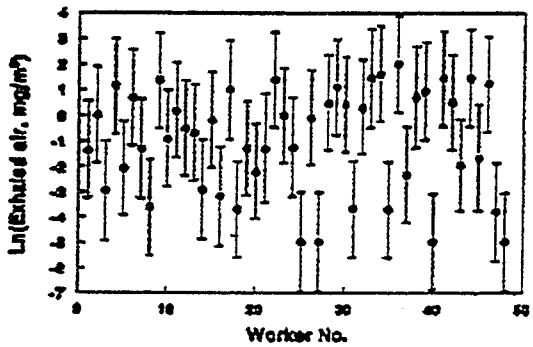
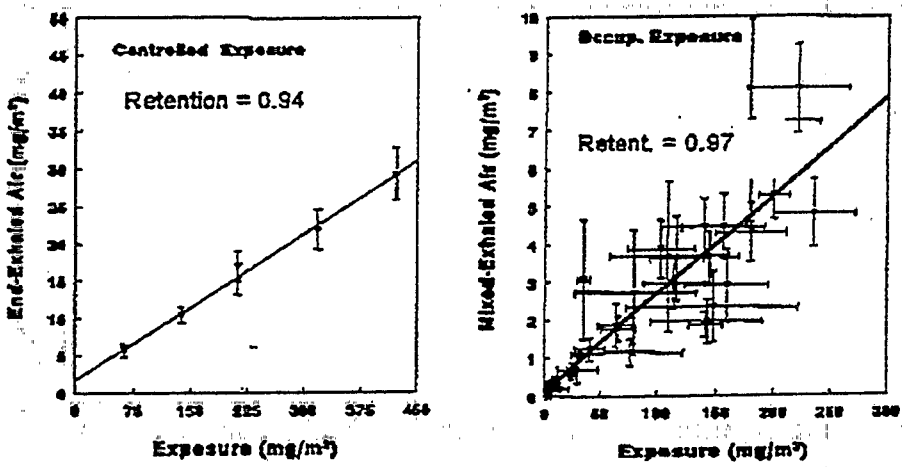


Figure 6-6

RETENTION OF STYRENE

TLV-TWA = 85 mg/m³

TLV-STEL = 170 mg/m³



Ref: Rappaport SM (1996) *Toxicol. Letters* 77:171-182. Based upon data from Petreas M, et al. (1996) *Int. Arch. Occup. Environ. Health* 67:27-34, and Yager J, et al. (1993) *Mutat. Res.* 319:155-165.

Figure 6-7

6.2 INTEGRATION OF APPROACHES

Louise Ryan, Harvard School of Public Health

Given data from an ethylene oxide developmental toxicity showing strong effects of duration for a given C × T fixed value (0, 2100, and 2400), how can one fit a model that describes the data well and allows extrapolation to regions not represented in the experimental design? Statistical approaches include:

- Fit a generalization of Haber's law model, e.g., $Y = \beta_0 + \beta_1 C \times T + \beta_2 T$
- Fit series of models using different dose metrics. Pick the "best" according to some goodness of fit standard (e.g., peak exposure, AUC, AUC above a threshold, etc.)
- Attach weights to different concentrations and sum over time (Allen Lefohn's approach)

The limitations of statistical approaches include the need for a lot of design points and the lack of reliability for extrapolating beyond the experimental design. A clear analogy exists with the classic problem of low-dose extrapolation.

Mechanistic approaches emphasize the following:

- Chemical properties
- Understanding metabolic and elimination pathways
- Identifying mechanisms of action
- Accounting for factors such as breathing rates
- Identifying the appropriate dose metric (blood or tissue concentration of parent chemical or metabolite, either peak or AUC)

Limitations of mechanistic approaches include the need for extra information that is not always measurable on the same animals, and the fact that mechanisms are not generally well understood.

To reconcile these perspectives, we can:

- Use biological information to inform decisions about the appropriate class of statistical knowledge.
- Use the biomarker paradigm.

To develop heuristics, consider the simpler problem of using a biomarker to develop a more accurate model for extrapolation to low dose. (Biomarkers or "quantal intermediates" are things in between the pathway from exposure to response; they can be indicators of the amount of exposure to the tissues, or of the amount of response in the tissue itself.) Figures 6-8 though 6-12 show a hybrid biological/statistical model using the biomarker paradigm.

Unifying the statistical and mechanistic models under the biomarker paradigm combines the best features of both approaches. Statistical methods to incorporate biomarkers into risk assessment are relatively undeveloped. Needs include a theory to properly account for uncertainty in predicting dose from exposure. Aspects of the measurement error framework apply.

Biomarkers in risk assessment

Consider

$$Pr(\text{Adverse event}|\text{exposure } X) = p(X)$$

Let Z be a biomarker (say GSH level) and

$$p(X, Z) = Pr(\text{Adverse event}|X, Z)$$

Function of interest, $p(X)$, obtained by integrating over the distribution of the biomarker:

$$p(X) = \int_Z p(X, Z) f(Z|X) dZ$$

What will $p(X)$ look like?

Figure 6-8

A special case

Consider version of the 1-hit model:

$$p(X, Z) = 1 - \exp(\alpha_0 + \alpha_1 X + \alpha_2 Z).$$

Integrating over distribution of Z yields:

$$\begin{aligned} p(X) &= \int_Z \left(1 - e^{(\alpha_0 + \alpha_1 X + \alpha_2 Z)}\right) f(Z|X) dZ \\ &= 1 - e^{(\alpha_0 + \alpha_1 X)} \int_Z e^{\alpha_2 Z} f(Z|X) dZ \\ &= 1 - e^{(\alpha_0 + \alpha_1 X)} \phi(\alpha_2, X) \end{aligned}$$

where $\phi(\alpha_2, X)$ is a function of X.

Depending on the relationship between Z and X, the function ϕ modifies the dose response model $p(X)$ to a generalization of the simple 1-hit model.

Figure 6-9

Maximum likelihood estimation

Likelihood contributions for three data settings:

A: Just X and Y (outcome)

$$\prod p(X)^{Y} \, [1 - p(X)]^{1-Y}$$

B: Just X and Z

$$\prod f(Z|X)$$

C: X, Z and Y

$$\prod p(X,Z)^{Y} \, [1 - p(X,Z)]^{1-Y} f(Z|X)$$

Classical measurement error formulation. Can estimate model parameters from (1) A alone or (2) A and B or C. Ideally all three, and the more C the better!

Figure 6-10

Notes and generalizations

- Statistical approach is A only
- Unlike biologically based models, does not assume mechanism fully explained.
  - If outcome is independent of X given Z, then mechanism is explained through Z (biological model)
  - If Z is independent of X, then Z is unrelated to mechanism (statistical model)
- X can be multidimensional (e.g. C and T)
- Z can be multidimensional
- Other methods besides maximum likelihood - regression calibration from measurement error context, Bayesian methods

Figure 6-11

More on C $\times$ T

Suppose we want to use information on GSH levels (Z) to construct $p(C,T)$, the dose response surface as a function of C and T

Assume:

$$p(C,T,Z) = 1 - \exp(\alpha_0 + \alpha_1 C \times T + \alpha_2 T + \alpha_3 Z)$$

Integrating over distribution of Z yields:

$$p(C,T) = \left(1 - e^{(\alpha_0 + \alpha_1 C \times T + \alpha_2 T)}\right) \phi(\alpha_3, C, T)$$

If α_1 and α_2 are zero, then Z "explains" the dose-rate effect. If α_3 is zero, then Z explains nothing and we are back to the statistical model

Figure 6-12

SECTION SEVEN

FUTURE DIRECTIONS: WHAT SHOULD BE ACCOMPLISHED IN THE NEXT FIVE YEARS?

The group discussed the merits of a range of steps to further clarify C × T issues. These included the following:

- Develop case studies, including some with less information but looking at a variety of mechanisms.
- Consider whether the dose metric could change with the same endpoint, the same mechanisms and the same chemical; consider where the point of activity is binding and the molecule has sites for multiple ligands that bind cooperatively. One might finds a model that fits but has nothing to do with the underlying mechanism. Hemoglobin-carbon monoxide is a possible choice, where the mechanism is understood.
- As we go below the whole-animal level, clearly designate what adverse effects are for mechanistic modeling.
- Look at pharmacodynamic modeling issues, thinking about how time works through those models, and about what principles are at work and how they can be simplified to more widely applicable pharmacodynamic models.
- Find ways to encourage researchers doing mechanistic work to routinely collect data related to C × T issues (e.g., examine endpoints at more than one dose or time). Place databases from published research on the Internet. A lot of mechanistic data exists but the studies are not designed to look at C × T issues.
- Show that EPA will use time-dependent data in extrapolations rather than the default (as is occurring with the HAPS rule); this will encourage industry to generate useful data in testing compounds.
- Encourage industry to expand their notion of product stewardship, so that in evaluating the cost-benefit of doing C × T for a certain chemical, determine if it will impact other chemicals in the same category.
- Ask those reviewing grant proposals to encourage researchers to generate C × T-related data.
- Encourage this research (e.g., case studies) in ORD solicitations.
- Determine how to incorporate these needs with constrained resources (e.g., one can gain dose groups by using 28 animals per dose group rather than 30, but if the required dose group size is still considered 30 in the risk evaluation, the tradeoff isn't worth it). EPA should consider balancing the C × T issue against statistical power of the number of animals at each dose.

- Think about data we already have in different ways (as in William Boyes's work—see Section 3.3, Figure 3-11); a lot can be learned by doing studies out of the literature.

- Ask industry to publish data already available. It would be important to identify the kinds of information needed and how it should be displayed.

SECTION EIGHT

SUMMARIES OF BREAKOUT GROUP DISCUSSION

This section summarizes the discussions that occurred in the breakout groups during one workshop. The groups were encouraged to discuss the topics without attempting to reach a consensus. Therefore, individual members of a group may agree or disagree with some of the specific statements in these summaries.

8.1 SUMMARY OF BREAKOUT GROUP ONE DISCUSSIONS

Resha Putzrath, Discussion Leader; Rory Conolly, Rapporteur; William Boyes; Harvey Clewell; Gary Kimmel; Paige Williams

8.1.1 Relationship Between Concentration and Exposure Duration (C × T) and Toxic Endpoint

The group discussed what issues should be considered in examining the relationship between the pattern of exposure and response, i.e., whether the $C \times T = constant\ response$ relationship is valid (or sufficiently valid). The following were considered potentially important:

- Whether the endpoint was reversible or not reversible can affect the relationship.
  - Reversibility (of pharmacokinetics or due to repair of damage) implies that the exposure history doesn't matter. This may mean that the momentary concentration at the target tissue is more important than the exposure history.
  - If the dose rate is greater than the repair rate, $C \times T = constant$ may apply.
  - Above saturation or below a threshold, $C \times T = constant$ is not interpretable. The assumption that $C \times T = constant$ implies a no-threshold model.
- Accommodation or tolerance by the animal or system is likely to change the relationship between exposure and toxicity.
- Whether the chemical is rapidly cleared or biopersistent will affect whether some patterns of exposure depart from $C \times T = constant$.
- Different relationships among exposure, concentration, and response might exist at different dose levels.
- Different relationships among exposure, concentration, and response might exist for acute exposure versus chronic exposure to the same chemical.
- Any nonlinearities in the dose-response relationship are important in evaluating the potential for departure from $C \times T = constant$.

The duration of exposure itself might affect the dose-response function. Over 8 hours during a developmental study, the biological entity can be changing rapidly, i.e., major changes in the structure and function of the organism can occur. Thus, during one exposure the entity has significantly changed.

- What does it mean to average exposure over those 8 hours? Should the comparison be to a 1-hr exposure at eight times the concentration at the beginning, middle, or end of this time?
- Do all 1-hr intervals need to be explored (cf. limitations on funding and other resources, below)?
- Even though the organism changes, the mode of action may or may not be affected.

The group discussed the changes in experimental design that would be necessary to explore the relationship between duration or pattern of exposure and toxicity. Procedures that might enhance our understanding prior to additional experiments were also addressed.

- Experimental conditions for exploring the relationship between exposure duration and toxicity require examination of numerous different combinations of concentration and time, including where $C \times T = constant$.
  - Such research is expensive, and funding is a concern.
  - Usually, it will be too expensive to determine empirically the shape of the complete C × T surface for adverse endpoints.
  - The experimental design might differ depending on whether the goal is understanding the potential toxicity of the chemical over a full range of doses or establishing a regulatory limit.
  - Should intermittent exposures be examined in addition to continuous exposures?
  - We should consider using our resources to define mechanistic endpoints that determine relationships between exposure, concentration, and response. Mechanistic data can be used to develop models of the relationship between C and T.
  - We should encourage editors of journals to encourage authors to publish data that they already have from their experiments which would assist in resolving these issues.
    - What information is likely to already be available from existing experiments that would be most useful for risk assessment?
    - Should these data be available through the Internet?
    - What are the implications of archiving data electronically, given changing software/hardware?
  - How could scientists be encouraged to include some additional data points in their experiments that would help resolve some of the issues regarding exposure pattern and toxicity? Is there guidance we can provide prior to experiments as to what (limited) additional information would be most useful for resolving this issue?

- The relationship between pattern of exposure and response will be a larger issue in the future. We need to think about the issues now.

- We might look to the pharmaceutical literature, in which it has been speculated that there might be more examples of relationships between exposure duration and concentration resulting in specific toxic effects.
  - Pharmacologists, however, are typically concerned with a rather narrow range of responses. The lower response levels of interest for regulatory purposes may not have the same relationship.
  - Some data exist in the literature, but full sets are unlikely for most chemicals.
  - One observer reported that for one database of 3,900 papers on 40 chemicals, only 6 had experiments that varied both concentration and time.
  - Are data available from industry, such as the data obtained from the successful call for data for examining the benchmark dose procedure?
- What might we do given limited resources?
  - Start with a conceptual model and design experiments to test predictions.
  - Use information about related chemicals that have been more extensively studied.
  - Characterize those areas of dose-response surface of regulatory interest and/or of use to validate preliminary model, not whole C × T grid.
- With regard to establishing relationships between exposure duration and time, the use of very high doses in laboratory animal experiments may be of questionable relevance for human exposure.
  - Relationships seen at high doses may not hold for doses of interest in risk assessments.
  - Models may predict unexpected effects in low-dose region, e.g., the preliminary model for the heat shock protein appears to predict responses below the control level at some low temperatures.

8.1.2 Mechanistic Modeling

The group discussed how mechanistic models can significantly enhance our understanding of the interaction between exposure duration, concentration, and response, as rate constants are usually built into mechanism-based models.

- The distinction between statistical and mechanistic models is not absolute. We are always limited by data gaps and will use empirical or statistical procedures to fill those gaps. Similarly, the choice of statistical model is often based on some assumptions about mechanism.

- Experimental data are needed to develop and validate the models. Developing mechanism-based models depends on an understanding of the mechanism. It would be useful to develop guidance for the data needs of mechanism-based models.

- PBPK modeling allows us to sort out the pharmacokinetics. This is an essential basis for sorting out the pharmacodynamics. Both are needed, and we need to start developing both, and iteratively work toward the center.
- Mechanistic research and development of models is expensive. If we want to be able to use it in risk assessment, someone will have to pay for it.

- Mechanistic models don't fully describe mechanisms, so they have correlational components where parameters are adjusted so that the model fits the data.

- How do we determine the confidence or reliability of the values produced by PBPK modeling? What if different models exist for the same chemical? Should it matter if they produce similar answers? How do we determine what is "similar" for scientific and/or regulatory purposes?

- How do we incorporate information from human exposure, including epidemiology?

- How do we incorporate human variability into mechanistic models?

- We need to consider positive effects as well as negative effects, e.g., tamoxifen, essential elements).

- What is the threshold for scientific peer acceptance of mechanistic modeling for risk assessment? Is it the same for regulatory acceptance?

The group discussed the differences between mechanism of action and mode of action. Without attempting to craft definitions for both terms, the group settled on a functional use of mode of action, i.e., that knowledge of it would provide sufficient information about the essential features of mechanism to be useful for risk assessment. The goal is sufficient information from the mode of action to define the overall shape of the dose-response curve (or surface).

- The mode of action determining the dose-response curve might change from the exposure where data are available to the exposure range of interest.

- Biochemical endpoints might be used to assist in evaluating the mode of action and thus toxicity.

  - Among biochemical changes, we have to define what is bad (adverse).

  - We need to recognize that there is a range of normal and that increases and decreases are possible.

  - It is necessary to determine that the endpoints we can measure are accurately correlated with the endpoint of interest.

8.1.3 Statistical Modeling

Increased availability of computational resources allow exploration of more "what if" questions. Results from such studies can enhance our understanding of when significant departures from $C \times T = constant$ may occur and how to design experiments to determine the relationship between exposure duration, concentration, and toxicity.

- Computational resources are not a barrier. In contrast, experimental resources, e.g., number of animals, can be an issue.

- Statistical modeling can be used to assist in the design of experiments for the more efficient use of animals (e.g., Williams, Kimmel). Pilot studies can be used in conjunction with statistical modeling to target the best use of resources, e.g., where on the dose-response surface interesting changes may occur.

- Statistical modeling suggests that just using the most sensitive endpoint may be anticonservative. Therefore, models that include all data (i.e., simultaneous effects) should also be considered.

  - The dose-response for each endpoint should be evaluated before attempting to combine the information.

  - Only statistically and toxicologically significant changes should be considered for combination.

  - More endpoints are becoming of interest to regulatory agencies. How should we determine how many (and which) should be combined into one risk assessment?

8.1.4 Dose Metric

Whether the $C \times T = constant$ relationship holds may depend on the metric by which both are measured.

- Dose metric can vary for the same chemical with a change in endpoint due to different modes of action.

- The dose metric can change with dose or duration, e.g., the accumulation of manganese over time results in a change in mode of action.

- Are we concerned with evaluating the relationship between exposure duration and concentration at the level of exposure or at the concentration at the target tissue?

- Should we consider models/metrics where there is an exponent on time, e.g., instead of $C^n \times T$, why not $C \times T^n$?

- Dose metric might change with dose, for example, if the mode of action involved (cooperative) binding of more than one ligand to an active molecule, where number of ligands bound affected V_{max} or K_m.

- A PBPK model might suggest the appropriate dose metric.
- Can various dose metrics be used as surrogates for modeling when data are limited, e.g., when we have information on dose metric for a related chemical?
- Use of the area under the curve (AUC) above a critical concentration implies a threshold.
- Various dose metrics should be considered in statistical models. The results of such evaluations can aid in the design of future experiments to explore the relationship between C and T.
- Use of the same dose metric from laboratory experiments in animals for human exposure assumes that humans and animals are qualitatively similar with regard to pharmacodynamics.
- Selection of a particular dose metric in a statistical exposure-response model has the advantages and disadvantages of both the mechanistic and statistical modeling approaches. This approach can be viewed as midway on the statistical-mechanistic continuum.
- It is important to acknowledge that there may be alternative plausible models.
  - We need to examine many models, as the same data may fit various models if different dose metrics are used.
  - Assumptions about dose metric, given sufficient fit of the data, may influence future experimental design. Prior assumptions can affect models, e.g., models where the planets moved around the Earth also fit the data sufficiently well.
  - It can be particularly difficult to distinguish among models when data are limited.
  - Pilot studies may assist by eliminating some models from further consideration.

8.1.5 Risk Assessment

The current default assumption by EPA is that $C \times T = constant$. Given that a model exists and is being used, what is required to change the model? Regulators tend to be comfortable with what is being done and often require a high level of proof for change. The level of proof may be greater if higher levels of exposure would be allowed, consistent with the desire to err on the side of protection. We should consider an *a priori* determination of what proof will be required, e.g., to demonstrate that the current model is not valid or that another model is more valid. For the purpose of changing a default in general or in a particular case, is there a need to distinguish between the new model being "correct" and its being "less wrong" than the current model, i.e., is it sufficient to establish that the proposed model is very likely to be more accurate than the current model?

- The $C \times T = constant$ adjustment should be recognized as a default with advantages but limitations.
- When can changes from the $C \times T = constant$ assumption be justified if we don't have, at a minimum, a good dosimetry model?

- The premise of the workshop, the draft paper, and most of the participants is that $C \times T = constant$ is the current procedure used. Some potential exceptions that may be already employed were mentioned.

  - Exposure may be adjusted to internal dose based on amount absorbed across a barrier (e.g., skin) or presence of other materials (e.g., food for oral exposure). This returns to the issue of whether the concentration is measured as exposure or dose to the target tissue.

  - Interspecies extrapolations may be based on internal dose.

  - RfDs and RfCs assume that a threshold exists. How do we accommodate the $C \times T = constant$ adjustment below a threshold?

- When should we invest resources to investigate C × T relationships?

  - Generally, we don't need to worry about deviations from the default assumption if the extrapolation away from the data is small. The question was then raised as to how to define "small".

  - Clear evidence for departure from linear metabolism might be the basis for assuming a departure from standard $C \times T = constant$.

- How can we provide guidance for experimental design and/or reporting of available data that would be useful for other risk assessment purposes than those of interest to the experimenter?

- A narrative, such as that being proposed for cancer risk assessment, might be useful for providing information about issues on how exposure patterns can affect toxic responses.

  - A discussion could indicate the effect on the risk estimate of selecting among various models or dose metrics (and their associated assumptions).

  - The amount of confidence in various choices could be presented.

- Many of the issues raised might be better examined with some case studies to deliberate.

8.2 BREAKOUT GROUP TWO

George Rusch, Discussion Leader; Edie Weller, Rapporteur; Dan Costa; Dale Hattis; Harvey Richmond; Louise Ryan

8.2.1 Endpoints of Toxicity

To generate discussion, the discussion leader described an experiment of dimethyl-carbonyl chloride in which animals were exposed via inhalation for 180×6 ppm-hr, 30×6 ppm-hr/5 days and 1×6 ppm-hr/1 year. The lethal concentration for each of these was 180 ppm-hr, 150 ppm-hr and 220 ppm-hr, respectively. Most animals at 180 ppm-hr died of irritation whereas most animals at 220 ppm-hr died of cancer. Therefore, we have two different endpoints of toxicity depending on the exposure pattern, while the total dose is almost the same.

This example generated discussion not only about the endpoints of toxicity but also about the mechanism of action, modeling, and the relationship of Haber's Law to the endpoints. The group discussed whether a threshold exists in this setting. It was proposed that if there is irreversible damage one would not expect a threshold biologically, whereas if there was reversible damage a threshold could potentially exist. It was also proposed that if there was no saturation in the system, one would expect Haber's model to hold. Members of the group expressed several opinions on these questions.

The group discussed endpoints for respiratory toxicity. It was proposed that body temperature and heart rate may be reliable endpoints for monitoring the effects from exposure over time because they reflect the direct stress on the animal. It was also mentioned that in inhalation studies it is important to account for ventilation rate when computing the internal dose. It was mentioned that the type of analysis used for these studies could be complicated if the animals are exposed repeatedly over time contrasted to a single high-level exposure, since in the first case one would have to consider the body's potential to adapt. An example was provided using sunburn as the endpoint. People initially exposed to the sun will burn. If they wait a few weeks, they will burn just as badly. If they are exposed to the sun again immediately, they will not burn as badly. The body's ability to adapt changes the toxicity response to the exposure. This means one may not have the same effect on the endpoint if exposed repeatedly over time—especially if these time points are close. This is an important characteristic to consider when defining the endpoints of toxicity.

The group also discussed endpoints for developmental toxicity. The endpoints that would be expected to be observed depend on the timing of the exposure during the gestational period. Therefore, the specific day of exposure often is more important than the total dose received over several days.

Based on the discussions the group proposed a family of equations that would explain the concentration-time relationship. The type of equations used will depend upon the type of exposure (e.g., repeated/continuous exposure versus one time) and the timing of exposure (e.g., timing during the gestational process in a developmental toxicity study). It was pointed out that the form of the model is specific to the endpoint considered. In addition, the group discussed the importance of mechanistic information for low dose extrapolation.

In discussion about the appropriateness of Haber's model, $R = \alpha C^n \times T$, there was disagreement within the group as to whether this truly holds for a specific biologic phenomenon. The exponential model was suggested as an alternative, potentially more biologically based model. An example with ozone was mentioned where the diffusion process tends to follow the exponential model.

The key points mentioned during the session included:

- Toxicological endpoint is important in determining the model
- Points to consider when choosing the endpoint
  - threshold
  - saturation
  - developmental
  - systemic
  - adaptation
  - reversibility

- Importance of mechanistic information for low-dose extrapolation
- Appropriateness of the $R = \alpha C^n \times T$ model versus the exponential model
- Need for a family of equations to explain the C × T relationship
- Generally, brief high-level exposures are more harmful than long-term exposures with the same total dose.

8.2.2 Statistical Approaches

The ethylene oxide experiment done at the Harvard School of Public Health (Weller et al., submitted 1998) was used as a guide to discuss statistical approaches and how best to incorporate the mechanistic information into the models. In this study, animals were exposed via inhalation to various concentration and durations of EtO on gestational day 7 (see Table 8-1 below). The maternal and developmental effects were assessed using dose-response models to assess whether there was a difference in effects between short duration/high concentration exposures and long duration/low concentration exposures.

Table 8-1
Experiment Design For EtO Experiment

ppm-hr	Concentration (C)	Duration (T)
0	0	1.5
	0	3.0
	0	6.0
2,100	1,400	1.5
	700	3.0
	350	6.0
2,700	1,800	1.5
	900	3.0
	450	6.0

Assuming that the mechanism of action and the correct mechanism of action were defined, the family of equations f_1 and f_2 were proposed. The first equation relates the concentration and times along with the other mechanistic information to the appropriate internal dose metric as

$$f_1(C,T, \textit{other variables}) \rightarrow \textit{Dose Metric}$$

and the second equation

$$f_2\,(\textit{Dose Metric}) \rightarrow \textit{Toxic Endpoint}$$

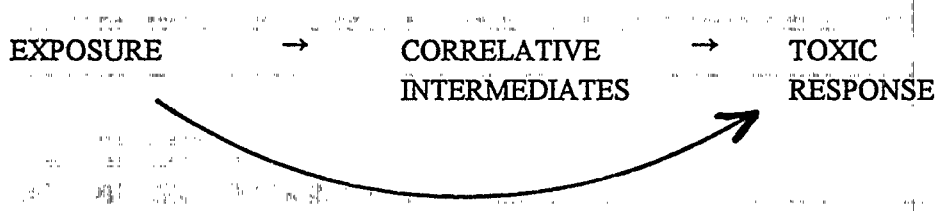
relates the dose metric along with other potential covariates that might affect a toxic response allowing for the uncertainty in the dose metric. This approach would use concepts similar to the measurement error methodology. The group discussed whether specific mechanistic information could be chosen that would most influence the amount of chemical that is ultimately absorbed in the body, and therefore, potentially be associated with a toxic response. The consensus from the group was that this approach seemed reasonable; however, there was some disagreement about the appropriate mechanism of action. The issues of statistical design were discussed briefly. The design characteristics that were preferred were those with the most precision of the coefficient of the dose metric in model f_2 and those with the most accurate risk assessment.

8.2.3 Dosimetry and Mechanistic Modeling

During this session the importance of selecting the correct dose metric and understanding the mechanism of action was stressed. An issue was raised about whether there is a need to understand everything, i.e., the whole black box, or whether some of the mechanistic information could be used in the approach proposed in Section 8.2.2. This could include, for example, information on the absorption and distribution of the chemical and the receptor activation, DNA damage, and cell death due to the chemical. The group agreed that it might not be necessary to understand the complete mechanism. An understanding of the details that would allow an understanding of time/dose dependency would be adequate. Again, the ethylene oxide study was used as a guide in these discussions.

8.2.4 Implications for Risk Assessment

The difficulties in risk assessment with respect to low-dose extrapolation and across-species extrapolation were discussed. The question was raised again as to whether all of the mechanistic information needs to be known to provide more information to predict what to expect in the low-dose region of C×T design. The following paradigm was proposed:



where all the mechanistic information may not need to be known but that some correlative/causal intermediates would be known. These intermediates would potentially provide adequate information about the effect of exposure on the toxic response and provide reliable estimates of the predicted toxic effect in the low-dose region.

Most of the subsequent discussion focused on a recommendation for immediate action that could be proposed to the workshop participants to resolve some of the issues related to studying the C×T relationships. The group developed the following recommendation:

- Look carefully at research that has been done on chemicals that are fairly well studied, such as EtO, formaldehyde, and ozone.
- Determine what needs to be done to fully study the C×T relationship for these chemicals.
- Apply the methodology proposed in the Statistical Approaches session (Section 8.2.2) to the data from these chemicals.
- Establish the appropriateness of the proposed approach.

The group stressed the importance of interdisciplinary communication in determining the appropriate correlative intermediates and the importance of focused research.

8.3 BREAKOUT GROUP THREE

Lorenz Rhomberg, Discussion Leader; Ronald Wyzga, Rapporteur; Annie Jarabek; Allen Lefohn; James McDougal; Stephen Rappaport

8.3.1 Dosimetry and Mechanistic Modeling

Mechanistic models should demonstrate qualitative agreement with biological understanding and quantitative agreement with existing data. It is important that application of these models provide some quantitative indication of how well they fit all of the existing data. Some measure of uncertainty should be given to the estimated parameters and projections made from these models. Often applications of these models do not distinguish between interpolation and extrapolation; it is important that such distinctions be made because of the greater uncertainty associated with extrapolation. Mechanistic models can be particularly valuable in undertaking sensitivity analyses to help indicate those variables/parameters that may be of greatest importance in influencing model results. Future experiments can then focus upon obtaining more and better data/estimates for these variables/parameters.

To date these models have largely been built using animal data. There is a strong need to develop human data that can be used to define these models. The result will be more confidence in using these models for health risk assessments.

There are two major components of these models: pharmacokinetics models and pharmacodynamic models. To date there has been considerably more experience in developing and applying pharmacokinetics models, and the value of these models is well-established. Pharmacodynamic models have not been well explored. There is no reason to believe that such models are intractable, and resources need to be made available to encourage the development and application of these models. This can only facilitate and improve the risk assessment process.

There are two modeling approaches: statistical/empirical models and mechanistic models. Each has its virtues and its disadvantages. It will be important to take advantage of the positive aspects of each type of

model and the marry them to the fullest extent possible. Close collaboration between biologists and statisticians will be required in this effort, as will additional experiments, which must be planned by both types of researchers.

Cross-species extrapolation is likely to vary with both concentration and duration; i.e., the same extrapolation factor may not be equally valid for different exposure-duration combinations. These factors can also vary by level of organization (e.g., cellular level vs. organ level) within a species; moreover, the pharmacokinetics and pharmacodynamic components associated with dose response may involve very different cross-species extrapolation factors. An understanding of these, however, may facilitate the choice of factor for different dose-response combinations and levels of organization.

It should be noted that the choice of dosimeter used in experiments or in modeling does imply some judgment about mode of action. In cases of greater uncertainty alternative metrics should be considered.

We *need* human exposure profile data. These data are very limited. The presence of these data could greatly influence the choice of exposures in experimental protocols. Models could also be run (see the presentation by Hattis in Section 4.1) to indicate how their results may vary with alternative exposure profiles. These can be very complex; for that reason, it is important to ensure that model considerations reflect reality and that ensuing regulations are indeed protective (and not overly protective) for the exposures likely to be encountered by real people in the real world.

Human ambient exposures are likely to be relatively low. At these exposures, pharmacokinetics are likely to be linear; hence these considerations may not be particularly important. Pharmacodynamic considerations, such as tolerance, may be particularly important at these exposures. Hence these need special consideration in risk assessments to be applied to ambient conditions.

8.3.2 Risk Assessment

Clearly more research is needed on the concentration-duration issue. There is general agreement that we want more accurate assessments. These need to lead to regulations protective of public health, but not overly protective to the extent that large costs are incurred with little marginal public health benefit. It is assumed that current regulatory approaches for risk assessment are conservative and protective of public health. If anything, it is assumed that conservative assumptions are made to account for uncertainties associated with our understanding of the exposure-health response relationship. This may not necessarily be true. In some cases, particularly when extrapolating from chronic exposures to acute exposures, the current approach may not be as protective as assumed. In any case better data/models will lead to more accurate risk assessments and to regulations based on more science and less judgment. More accurate risk assessments and less uncertainly will, in general, lead to less need for regulatory agencies to apply conservative safety factors or models to set regulations, yet regulations can be equally protective. The increased information will simply indicate that conservative assumptions need not be applied because there is less uncertainty. The possibility of less onerous regulations should then provide incentives for those being regulated to support the generation of better data and models. To ensure that this cycle operates well, participating parties need to operate in good faith. Regulatory agencies need to be willing to relax regulations when less uncertainty allows them to and they need to communicate this fact to the regulated community, and the regulated community needs to take steps to ensure that their research is of the highest quality and can withstand peer scrutiny.

If C × T, pure and simple, does not work (is not appropriate), then resulting risk assessments become more problematic. Current default methods often assume a simple C × T relationship. The inappropriateness of this assumption can result in both overestimates and underestimates of risk, with the latter being more likely when extrapolation is toward periods of shorter duration. It is important that the definitions of C and T be clear. Regulations target external or ambient levels of C; biological definitions can vary considerably from the concentration at the point of intake to the target organ concentration. The validity of the formula clearly will depend upon the definition used, and when the best definition of C is not the regulatory definition, appropriate adjustments need to be made. Similarly, alternative definitions of T are possible. Some regulations allow complete flexibility in the time average for a regulation (e.g. NAAQS); in these cases a regulation can be set for the time averaging period that demonstrates adverse response in health research. Other regulations do not appear to be as flexible (this could be by tradition or practice); flexibility is urged. It is better to have regulations set for time averages for which we have health data than to set a regulation for one time period with the intention of protecting for effects that occur at a very different time period.

Alternatives to the simple use of C × T require more information, which in turn can require money and more time. If additional information is available, clearly it should be used to derive appropriate improvements to the duration extrapolation procedure. If time and money are not of the essence, more data can be generated to increase the confidence and precision of duration extrapolations. In some cases "reasoned guesses" can be made about the appropriateness of the simple C × T relationship. For example, if a great deal is known about one chemical, it may be possible to make inferences about another chemical. In other cases it may be possible to suggest how pharmacokinetics may alter the relationship at the exposures of concern. In all cases, including the default assumptions, it will be important to articulate the assumptions and uncertainties associated with the resulting risk assessment.

APPENDIX A

WORKSHOP AGENDA

United States
Environmental Protection Agency
Office of Research and Development

Workshop on the Relationship Between Exposure Duration and Toxicity

Sheraton Crystal City
Arlington, VA
August 5-6, 1998

Final Agenda

Workshop Chair: **Louise Ryan, Harvard School of Public Health, Cambridge, MA**

WEDNESDAY, AUGUST 5, 1998

8:30AM **Registration/Check-In**

9:00AM **Welcome Remarks and General Introduction**
Louise Ryan

9:10AM **Introduction of Invited Experts**

9:30AM **"CxT": Historical Perspectives, Current Issues, and Approaches**
Annie Jarabek, EPA/National Center for Environmental Assessment (NCEA), Research Triangle Park, NC

10:30AM BREAK

10:45AM **Plenary Presentations: Endpoints of Toxicity**

Developmental Toxicity
Gary Kimmel, EPA/NCEA, Washington, DC

Dermal Toxicity
James McDougal, Geo-Centers, Inc., Wright Patterson Air Force Base (AFB), OH

Neurotoxic Effects of Trichloroethylene Inhalation as a Function of Exposure Concentration and Duration, Target Tissue Dose
William Boyes, National Health and Environmental Effects Research Laboratory (NHEERL), Research Triangle Park, NC

Respiratory Toxicity
Dan Costa, NHEERL, Research Triangle Park, NC

11:45 AM **Observer Comments**

12:00 NOON LUNCH

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WEDNESDAY, AUGUST 5, 1998 (Continued)

1:00 PM **Breakout Group Discussions: Endpoints of Toxicity**

Group 1 Discussion Leader: *Resha Putzrath, Georgetown Risk Group, Washington DC*
Group 1 Rapporteur: *Rory Conolly, Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC*

Group 2 Discussion Leader: *George Rusch, AlliedSignal, Inc., Morristown, NJ*
Group 2 Rapporteur: *Edie Weller, Harvard School of Public Health, Cambridge, MA*

Group 3 Discussion Leader: *Lorenz Rhomberg, Harvard School of Public Health, Cambridge, MA*
Group 3 Rapporteur: *Ronald Wyzga, EPRI, Palo Alto, CA*

2:30 PM **Plenary Presentations: Statistical Approaches**

What Can Mechanisms Tell Us About Modeling Dose Time Response Relationships?
Dale Hattis, Clark University, Worcester, MA

CxT Issues Related to National Ambient Air Quality Standards (Eco Effects)
Allen Lefohn, ASL & Associates, Helena, MT

Statistical Models for Assessing Dose-Rate Effects
Paige Williams, Harvard School of Public Health, Cambridge, MA

3:30PM **Breakout Group Discussions: Statistical Approaches**

Group 1 Discussion Leader: *Resha Putzrath*
Group 1 Rapporteur: *Rory Conolly*

Group 2 Discussion Leader: *George Rusch*
Group 2 Rapporteur: *Edie Weller*

Group 3 Discussion Leader: *Lorenz Rhomberg*
Group 3 Rapporteur: *Ronald Wyzga*

5:00PM ADJOURN

THURSDAY, AUGUST 6, 1998

8:30AM **Plenary Review of Breakout Group Discussions**

9:30AM **Plenary Presentations: Dosimetry and Mechanistic Modeling**

Dosimetry: Mechanistic Determinants of Exposure-Dose-Response
Annie Jarabek

Dosimetry and Mechanistic Modeling
Harvey Clewell, ICF Kaiser, International, Ruston, LA

10:15AM BREAK

THURSDAY, AUGUST 6, 1998(Continued)

10:45AM **Breakout Group Discussions: Dosimetry and Mechanistic Modeling**

Group 1 Discussion Leader: *Resha Putzrath*
Group 1 Rapporteur: *Rory Conolly*

Group 2 Discussion Leader: *George Rusch*
Group 2 Rapporteur: *Edie Weller*

Group 3 Discussion Leader: *Lorenz Rhomberg*
Group 3 Rapporteur: *Ronald Wyzga*

12:00NOON LUNCH

1:00PM **Plenary Presentations: Implications for Risk Assessment**

Implications for Risk Assessment
Steve Rappaport, University of North Carolina, Chapel Hill, NC

Integration of Approaches
Louise Ryan

1:45PM **Breakout Group Discussions: Implications for Risk Assessment**

Group 1 Discussion Leader: *Resha Putzrath*
Group 1 Rapporteur: *Rory Conolly*

Group 2 Discussion Leader: *George Rusch*
Group 2 Rapporteur: *Edie Weller*

Group 3 Discussion Leader: *Lorenz Rhomberg*
Group 3 Rapporteur: *Ronald Wyzga*

2:45PM **Observer Comments**

3:00PM **Plenary Review of Breakout Group Discussions**

3:30PM **Future Directions: What Should be Accomplished in the Next 5 Years?**
Moderated by Louise Ryan

4:00PM **Closing Remarks**

4:15PM ADJOURN

APPENDIX B

CHARGE TO PARTICIPANTS

United States
Environmental Protection Agency
Office of Research and Development

Workshop on the Relationship Between Exposure Duration and Toxicity

Sheraton Crystal City
Arlington, VA
August 5-6, 1998

CHARGE TO THE PARTICIPANTS

Background

Current risk assessment procedures are typically based on overall daily exposure levels, and tend to emphasize effects resulting from continuous exposures over a lifetime. However, there has been an increasing realization that exposures are more likely to be experienced as bursts or spikes, or intermittent exposures of varying levels. The Agency's Risk Assessment Forum is beginning to examine how dose-duration relationships are or can be incorporated into the risk assessment process for less-than-lifetime exposures. As part of this effort, the Risk Assessment Forum, together with the Harvard School of Public Health, is organizing a workshop to discuss our current understanding of dose-duration relationships, the approaches that can be used in their modeling, the inclusion of these relationships in risk assessment, and future directions in this area.

Charge to the Invited Participants

The objective of the workshop is to provide a forum for open discussion and to identify areas of consensus, as well as areas of difference. Approximately one week prior to the workshop, each participant will receive a working draft of an issues paper and a list of breakout groups. The paper is intended to explore issues in the assessment of dose-rate effects in order to identify where the current risk assessment approach may be improved and to identify gaps in our knowledge and methodology in order to suggest areas of further research. During the workshop, several presentations will be made to provide specific examples of the various issues that are defined in the paper. Every invited participant is asked to read the issues paper prior to the workshop, and be prepared to discuss it and the issues addressed in the presentations during the breakout group and plenary session discussions.

Each participant will be assigned to a specific breakout group. In making the group assignments, the organizers seek to ensure a mix of expertise in each group. Each breakout group will have a discussion leader to facilitate the discussion and a rapporteur to capture the discussions of the group. It is important that each of you participate in the breakout group to which you have been assigned.

We look forward to your input and to a productive and enjoyable workshop.

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APPENDIX C

LIST OF INVITED PARTICIPANTS
INVITED PARTICIPANTS' BIOGRAPHIES
LIST OF EPA PARTICIPANTS
LIST OF OBSERVERS

United States
Environmental Protection Agency
Office of Research and Development

Workshop on the Relationship Between Exposure Duration and Toxicity

Sheraton Crystal City
Arlington, VA
August 5-6, 1998

Final List of Invited Participants

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United States
Environmental Protection Agency
Office of Research and Development

Workshop on the Relationship Between Exposure Duration and Toxicity

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August 5-6, 1998

Invited Participants' Biographies

HARVEY CLEWELL

As Senior Project Manager and Director of the Health Assessment Group at ICF Kaiser International Dr. Clewell is reponsible for directing all pharmacokinetic modeling activities, as well as providing expertise on the application of physiologically-based pharmacokinetic modeling in chemical risk assessment and pharmaceutical safety assessment. Prior to working for ICF Kaiser, Dr. Clewell was the Director of the Risk Assessment Program for ManTech Environmental Technology, Inc. at Wright-Patterson Air Force Base in Ohio. In this position, Dr. Clewell was responsible for directing all risk assessment activities in support of Air Force and Navy requirements, as well as providing expertise on chemical risk assessment to other corporate programs. Dr. Clewell has done graduate course work in computer science and mathematics at the Air Force Institute of Technology. He received his M.A. in physical chemistry from Washington University and his B.A. in chemistry from Bradley University. Dr. Clewell has authored numerous publications including: *Applying Simulation Modeling to Problems in Toxicology and Risk Assessment - A Short Perspective; Reanalysis of Dose-Response Data from the Iraqi Methylmercury Poisoning Episode; Tissue Dosimetry, Pharmacokinetic Modeling, and Interspecies Scaling Factors; Use of Physiologically-based Pharmacokinetic Modeling to Investigate Individual Versus Population Risk* and *Pharmacokinetic Dose Estimates of Mercury in Children* and *Dose-Response Curves of Performance Tests in a Large Epidemiological Study.* Dr. Clewell is a member of the American Chemical Society and the Society for Risk Analysis. He is a recipient of the Air Force Scientific Achievement Award and the Air Force Meritorious Service Medal.

RORY CONOLLY

For the past nine years Dr. Conolly has worked in the Risk Assessment Department and the Department of Inhalation Toxicology and Biomathematical Modeling of the Chemical Industry Institute of Toxicology (CIIT). Prior to his work at CIIT, Dr. Conolly worked in the Toxic Hazards Research Unit of NSI Technology Services Corporation. Dr. Conolly received his Sc.D in physiology (toxicology) and his B.A. in biology from Harvard University. Dr. Conolly has received the Outstanding Presentation in Risk Assessment Award at the Annual Meeting of the Society of Toxicology. He is the Vice President of the Risk Analysis Specialty Section of the Society of Toxicology. Dr. Conolly's publications include: *A Physiologically-Based Pharmacokinetic Model Describing 2-Methoxyacetic Acid Disposition in the Pregnant Mouse; Cancer and Non-Cancer Risk Assessment: Not So Different If You Consider Mechanisms; A Strategy for Establishing Mode of Action of Chemical Carcinogens as a Guide for Approaches to Risk Assessments; Improvements in Quantitative Noncancer Risk Assessment* and *Implementation of EPA Revised Cancer Assessment Guidelines: Incorporation of Mechanistic and Pharmacokinetic Data.*

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DALE HATTIS

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Workshop on the Relationship Between Exposure Duration and Toxicity

Sheraton Crystal City
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August 5-6, 1998

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APPENDIX D

ISSUES PAPER

ISSUES IN EVALUATING THE RELATIONSHIP BETWEEN EXPOSURE DURATION AND TOXICITY

Sheraton Crystal City
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DRAFT

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Issues in Evaluating the Relationship Between Exposure Duration and Toxicity

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SUMMARY

Regulatory agencies have become increasingly aware of the need to develop exposure standards for varying lengths of exposure. Historically, the strategy for comparing responses among exposures of different durations has often relied on a relationship attributed to Haber (1924) that response levels should be equivalent for any constant multiple of dose times duration, i.e., whenever the cumulative exposure remains constant. This premise is widely recognized to be an over-simplification, yet may provide a good starting point for considering toxic effects of varying durations of exposures. To fully understand the complexities of exposure effects on toxic responses, one should ideally take into account the entire exposure profile, including the timing, duration, and intermittent nature of exposures reflecting realistic scenarios encountered in practical settings. The proper dose metric to characterize an exposure will be highly dependent on the pharmacokinetic properties of the chemical or exposure in question, and the toxic effects considered in models must likewise be carefully chosen to reflect the relevant endpoints based on the exposure and dosimetry characteristics. Models have been developed over the last few decades which begin to address the effect of duration of exposure in addition to exposure levels; however, many of these models do not incorporate mechanistic information. In addition, only limited work has been done on developing efficient designs for studying dose-rate effects, and these designs tend to be simplistic. The purpose of this paper is to explore issues in the assessment of dose-rate effects on toxic endpoints, by summarizing current defaults and identifying gaps in our knowledge and scientific methodology so that further research in this area can be focused and productive.

1 Historical perspectives and current default approaches

Current risk assessment procedures are typically based on overall daily exposure levels, and tend to emphasize effects resulting from low-level continuous exposures over a lifetime. However, there has been an increasing realization over the last several decades that exposures are more likely to be experienced as bursts of higher concentrations over shorter time periods, or intermittent (recurring) exposures of varying levels. In addition, data collected from subchronic studies are often extrapolated to develop estimates of noncancer risk, such as No Observed Adverse Effect Levels (NOAELs), for lifetime chemical exposures. Traditionally, a 10-fold uncertainty factor is applied when a subchronic exposure is used to estimate a chronic NOAEL. However, some feel that these extrapolated lifetime values may be overestimated (Crofton et al, 1996). Due to the fact that exposures are often experienced over shorter durations and at higher levels than the regulatory standards are intended to represent, understanding the underlying biological relationship between timing and duration of exposure and subsequent toxic effects is important in developing risk assessment strategies for various durations of exposures.

As Jarabek (1995) notes, much of the health assessment procedures have been based on a 1983 National Academy of Science (NAS)/National Research Council report (NRC, 1983) which recommends utilizing exposure scenarios as close as possible in practice to the standards they are meant to reflect. Thus, standards for acute toxic effects, such as 15-minute occupational time weighted average ceilings, are derived from animal experiments which are also classified as acute, such as 1 to 24 hour single inhalation exposures. Similarly, subchronic standards such as those resulting from periodic contaminations are usually defined on the basis of 90-day subchronic animal studies. Default assumptions are used in determining standards, so that the standards apply to a 70-kg male who drinks 2 liters of water and inhales 20 cubic meters of air per day. While the NRC report establishes a paradigm for setting standards based on different durations, it does not specifically address the issue of dose-rate effects.

In the context of incorporating duration of exposure, the use of animal studies to define exposure standards for human health assessment suffers from all of the usual limitations of cross-species extrapolation, pharmacokinetic differences in metabolism and distribution of the environmental agent, sensitivity and variability in response for different endpoints, etc. In addition, accounting for duration of exposure requires an additional assumption that toxic effects resulting from an animal experiment comprising a certain percentage of that species' lifetime would be comparable to the effects resulting in humans over the same percentage of lifetime. For example, the 2-year chronic animal bioassay study is considered to be representative of the effects over a 70 year human lifespan, and standards for a 7-year subchronic exposure in humans would be based on a 3-month subchronic animal study, each representing approximately 10% of the total lifespan. However, the timing of exposure is not considered in these default assumptions. While this may not present too many difficulties in assessing lifetime exposures, the point in chronological development may be a key factor in the response of an animal subjected to an acute or subchronic exposure.

There are a number of different regulatory statutes whose implementation activities

require consideration of exposure durations varying from as short as 15 minutes to an entire lifetime. These include the Clean Air Act Amendments of 1990, the Safe Drinking Water Act, the Clean Water Act, the Comprehensive Environmental Response, Compensation and Liability Act, the Resource Conservation and Recovery Act, and the Occupational Safety and Health Act. Some of the exposure limits set in response to these statutes are summarized in Table 3 of Jarabek (1995). For example, three different levels of "Emergency Response Planning Guidelines" (ERPG's) given a one-hour exposure duration are defined by the American Industrial Hygiene Association (AIHA) aimed at effects ranging from mild transient health effects to life-threatening outcomes. In some cases, the requirement for setting standards for specific exposure durations has led to a situation where data collected under one exposure scenario is utilized for exposures of a very different duration and level. For example, Weller *et al.* (1998a) note that exposure-response relationships for 189 air toxics have been derived primarily from acute lethality studies, but these results must be extrapolated to provide standards for ambient air environmental exposure levels, which tend to be much lower in magnitude. The lack of data from a range of exposure durations combined with the mandate to set realistic exposure standards has led to an increased need for more sophisticated quantitative models.

The development of quantitative risk assessment models has followed a premise generally attributed to Haber (1924) that toxicity levels should be linearly related to the product of dose level times duration, or "$C \times T$" (concentration x time). In its simplest form Haber's Law would imply that, for example, a one-hour inhalation exposure at 800 ppm should produce the same toxic response as an 8 hour exposure at 100ppm. In fact, Haber proposed this relationship in the context of evaluating very short-term effects of gas warfare, and did not suggest that it be used for extrapolating effects of exposure from very short term to long term. However, Haber's law has been used implicitly by some agencies like the Agency for Toxic Substances and Disease Registry (ATSDR) to adjust effect levels like NOAELs from animal studies to those of human exposures. For example, if animal studies for a particular exposure are conducted using 8 hour exposures and the human exposure of interest is 24 hours, then the effect level from the animal study would be divided by 3. In other words, it would be assumed that an exposure of NOAEL/3 for 24 hours would result in the same toxic effects as exposure to the NOAEL for 8 hours.

Bliss and James (1966) suggested that Haber's law tends to be most appropriate when evaluating either very short exposures to high concentrations or long chronic exposures to low concentrations. When Haber's law is violated, use of this premise for extrapolation purposes can lead to either underestimates or overestimates of the exposure levels associated with a particular toxic effect. While a great deal of attention has been addressed at documenting exceptions to Haber's Law, relatively less effort has been focused on extension of risk assessment methods, especially dose-response models, to account for duration of exposure.

Direct references to Haber's law have been cited most often in the context of animal experiments, yet this framework has also been used extensively by epidemiologists to account for the effects of exposures over various durations on human health outcomes. A typical strategy for representing exposure level is to use the concept of "person-time" exposure, which accounts for the length of time at risk for each individual and then sums these times

over all individuals in a cohort. The cumulative duration of exposure at a certain dose level is then assumed to be related to risk of adverse events. In a notable exception to this framework, epidemiologists studying the risk of radiation exposure have noted that long exposures at low dose levels generally produce lower risks than short intense exposures of the same total dose for low-linear energy transfer (LET) exposures, while for high-LET the reverse may be the case (Thomas, 1990; BEIR reports of 1980, 1990). In the area of radiation carcinogenesis, there have also been several investigations which support the dependence of cancer risk on the timing of exposure (i.e., age at exposure), time since exposure, and the duration of exposure.

For most exposures of interest, there is little epidemiological evidence available and risk assessment tends to be based primarily on experimental studies conducted in laboratory animals. These animal studies are conducted to characterize the dose-response relationship, but rarely address the effects of exposure over a range of exposure durations. There are some notable exceptions to this general statement, including work that has been conducted to investigate the relationship between exposure duration and toxicity on several different types of endpoints, including **dermal toxicity** (McDougal), **neurotoxicity** (Crofton et al, 1996; Bushnell, 1996 (abstract), 1997 (abstract), 1997; Bercegeay, 1997 (abstract), Boyes, 1996 (abstract), O'Shaughnessy and Losos, 1986; Hattis and Shapiro, 1990; Miller et al, 1983; Tilson and Cabe, 1979; and Yoshimura et al, 1992), **developmental toxicity** (Kimmel et al, 1993 (abstract), 1994 (abstract), Scharfstein and Williams, 1994; Williams, Molenberghs, and Lipsitz, 1996; and Geys, Molenberghs, and Williams, 1998), **inhalation effects** (Jarabek, 1995; Weller et al, 1998a, 1998b), and **vegetation effects** (Lefohn and Jones, 1986; Lefohn and Runeckles, 1987; Musselman et al, 1994). Further details of some of these studies will be discussed in the following sections.

2 Toxic Endpoints

The biological level of endpoints ranges from the whole animal and systemic endpoints to cellular-subcellular-molecular effects. Traditionally, regulatory agencies have relied primarily on effects at the whole animal/systemic level to define toxicity. This is not to say that examples of regulatory assessments which have considered endpoints at the cellular-subcellular-molecular level are non-existent, but rather that systemic effects have become the primary basis for most regulatory standards. In this context, exposure is defined on the basis of the whole animal rather than at the target issue (tissue/cellular), let alone the target site (cellular/molecular). Likewise, the endpoint (effect) is generally measured at the level of a systemic occurrence or event, rather than further distinguishing the cellular-subcellular-molecular effects. These approaches are in part a natural extension of test systems which have been developed and accepted with time. However, when considering concentration-duration relationships, an issue for discussion is what biological level can or should be assessed in establishing a toxicologic exposure? Is it necessary to have an understanding of the mechanism of toxicity? How do such tissue and cellular functions as repair, metabolism, and bioaccumulation factor into defining the relationship? Is it possible to adequately describe a concentration duration relationship without measuring effects at biological levels below whole animal-systemic?

The type of toxic endpoint of interest often depends on whether the exposure duration is acute (single dose), repeated in frequency, or chronic. However, the endpoint itself may not parallel the type of exposure. For example, single dose exposures may result in some immediate acute effects, such as irritation, hearing loss, CNS depression, or even lethality. However, they may also lead to chronic health effects if the single acute exposure leads to irreversible damage. Similarly, chronic lower-level exposures may result in both immediate acute effects after each exposure in addition to long-term chronic effects. For repeated exposures, the duration between exposures may allow for repair of damage and elimination of toxic metabolites, resulting in toxic effects of milder severity than those of single acute exposures. However, repeated exposures may also lead to bioaccumulation or may not allow sufficient time between exposure for repairing damage, so that chronic effects develop. Again, the choice of endpoints depends on an integral understanding of the mechanism of action of the agent in question, and the possibility that an exposure may result in several different types of endpoints must be considered.

Numerous examples exist of CxT studies carried out at the whole animal-system level. For example, several investigations have been conducted to evaluate the effects of repeated exposures to toxins. Investigations comparing the effects of repeated exposures of acrylamide with a single acute exposure have been conducted by Miller et al (1983) and Tilson and Cabe (1979). Burek et al (1980) have also studied possible repair mechanisms after exposure to acrylamide under various recovery periods. Bushnell et al (1997, abstract) examined changes in tolerance to repeated inhalation of trichloroethylene (TCE) in rats, and found that behavioral changes existed even after accounting for changes in metabolic tolerance.

Other whole animal or systemic studies have been conducted to address both qualitative and quantitative differences in toxic endpoints for short-term high-dose exposures versus longer term low-dose exposures. In the area of neurotoxicity, O'Shaughnessy and Losos (1986) compared CNS lesions in rats following acute high-dose exposures to low-dose chronic exposures. Yoshimura et al. (1992) investigated a number of different chemicals, and found that in many cases the same chemical led to different types of neurotoxic endpoints when administered to dogs and rats in high doses for short periods as compared to low doses for longer periods. Numerous studies conducted in the 1980's by Musselman et al (1983, 1986), Hogsett et al (1985), and Lefohn and Jones (1986) indicated that peak exposures to ozone and sulfur dioxide were more important in determining effects on vegetation growth than the total cumulative exposure. These studies led to development of exposure indices which placed greater weight on higher concentrations of ozone than on lower levels (Lefohn Benedict, 1982; Lefohn and Runeckles, 1987; Lee et al., 1988). Rappaport (1991) has also noted the importance of peak exposures in occupational exposure assessment.

In some instances, the endpoint of interest has been related to the administered exposure, while in other cases, correlations with target tissue levels have been found to be greater. For example, Crofton et al (1996) investigated the effect of dose rate on neurotoxicity of acrylamide by exposing rats to 1 day, 10 day, 30 day, or 90-day exposures, and found that the behavioral effects (motor activity, grip strength, and startle response) of acrylamide depended on both the level and duration of exposure. However, the recovery of behavioral

function was found to be independent of duration of dosing, and indicated that the toxicity was not due to an accumulation of acrylamide in the target tissue. Similarly, Boyes et al (1996), Bushnell (1996, abstract), Bushnell et al (1997), and Bercegeay et al (1997, abstract), have found that Haber's Law did not predict the relationship between TCE inhalation and changes in behavioral function in rats as measured by visual evoked potentials (VEPs), response time, or sensitivity index, but noted that the peak arterial concentration of TCE estimated by a physiologically-based pharmacokinetic (PBPK) model was a good predictor of all three of these neurologic endpoints, and tended to be more highly correlated with concentration than with the $C \times T$ product.

These and other studies have expanded our understanding of the limitations of Haber's conjecture, but the question remains as to how this understanding can result in predictive models of concentration-duration relationships that can estimate the potential for a given CxT multiple to be of concern. Moreover, the appropriateness of specific approaches to both acute-short term and chronic-long term exposures must be established. While risk assessment procedures have historically been developed to reflect lifetime exposures and have therefore emphasized chronic endpoints such as tumor development, a number of guidelines have been more recently developed for acute and shorter-term exposures. For example, within the EPA, the Office of Drinking Water develops health advisories for 1-day and 10-day consumption levels, and the Office of Prevention, Pesticides, and Toxic Substances addresses emergency responses to accidental releases of toxic substances and episodic exposures to pesticides. The guidelines have often been derived for specific populations; for example, the drinking water advisories are developed separately for adults and children, and some guidelines such as "EEGL's" have been developed with military personnel in mind. A summary of guidelines adapted from Kimmel (1995) and Jarabek (1995) and their relationship to various toxic endpoints is shown in Table 1.

As mentioned above, these guidelines and the test systems used to establish current exposure limits focus for mainly on the whole animal-systemic level of biological organization. This is in part due to a reliance on the traditional test systems that have been used in toxicology and the wealth of historical comparative data that is available. These test systems tend to have a clear definition of what response is considered to be a sign of toxicity, and they tend to be "apical", i.e., they examine the overall effect of an exposure on the biological system. As models of concentration-duration relationships are developed, it will be important to establish how compatible or complimentary they are with current approaches in risk assessment.

3 Dosimetry/Mechanistic Modeling

The issue of dosimetry is intrinsically linked to the effect of interest and the mechanism of action of the environmental agent. The appropriateness of applying Haber's Law, which in turn implies use of the cumulative exposure $C \times T$ as the the dose metric, depends on the pharmacokinetics of the particular agent and the dose/duration patterns under consideration. If the administered dose results in saturation, then higher doses may not have any effect on response rates regardless of the duration of exposure. On the other hand, if the pathway is not

Table 1: Basis for Short-term Exposure Guidelines

Guideline[1]	Organization[2]	Basis	Duration	Frequency
TLV-STEL	ACGIH	Irritation, impaired work, irreversible tissue damage	15 min	≤4x daily
TLV-EL	ACGIH	3 times TLV-STEL	30 min	recurrent
IDLH	NIOSH	Death, irreversible health effects	<30 min	one-time
ERPG	AIHA	Levels: (1) life threatening	1-hour	
		(2) irreversible, impair response	1-hour	
		(3) mild transient effects	1-hour	
EEGL	COT	Acute effects in military impairing emergency response	1-24 hours	constant
SPEGL	COT	Irreversible health effects	1-24 hours	constant
CEGL	COT	Irreversible health effects	90 days	constant
CEEL	COT	Irreversible health effects	1-8 hours	constant
HA	COT, EPA	Adverse health effects	1-10 days	one time

[1] TLV-STEL: threshold limit value, short-term exposure level
TLV-EL: threshold limit value, excursion limit
IDLH: immediately dangerous to life and health
ERPG: emergency response planning guideline
EEGL: emergency exposure guidance limit
SPEGL: short-term population exposure guidance limit
CEGL: community exposure guidance limit
CEEL: community emergency exposure level
HA: health advisory

[2] ACGIH: American Conference of Governmental Industrial Hygienists
NIOSH: National Institute of Occupational Safety and Health
AIHA: American Industrial Hygiene Association
COT: Committee on Toxicology of NAS

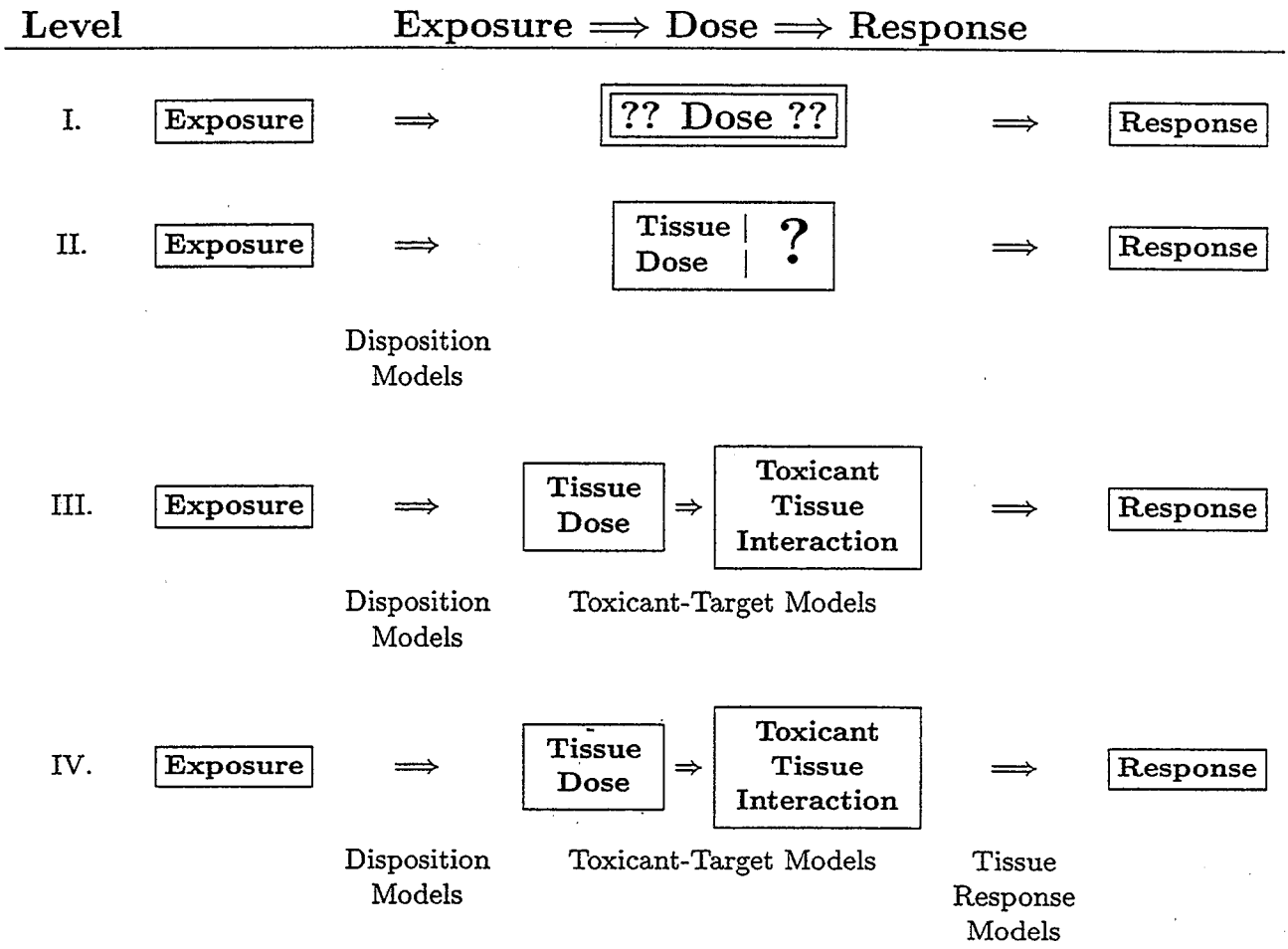
saturated and the dose delivered to the target organ depends on the metabolic rate through the area under the curve (AUC), then Haber's Law may provide a reasonable starting point for examining dose-duration response relationships. The latter condition will typically hold as long as the overall rate of elimination of the toxic substance follows a first-order process at the tissue of interest. Andersen *et al.* (1987) suggested that toxicity for most industrially important gases and volatile liquids would probably be related to the AUC rather than peak blood concentrations; in this case, use of $C \times T$ as the dose metric might be an acceptable basis for extrapolation. Jarabek (1995) notes that an alternative to using $C \times T$ as the metric is to use concentration alone regardless of duration, and points out that this might be appropriate for irritants for which damage does not accumulate with duration.

Figure 1 (adapted from Jarabek, 1995) reflects the various levels to which the exposure-dose-response continuum can be explored, and lists some of the mechanisms which might be elucidated to address relationships between external or administered exposure, dose at the tissue level, and response at the systemic, tissue, cellular or subcellular level. Level I reflects what is referred to as a "black-box" dosimetry model, in which the relationship between administered exposure and internal dose is unknown, but systemic toxic effects can be evaluated and related to the administered exposure. Level II reflects the inclusion of pharmacokinetic (disposition) models, which relate the exposure to the internal tissue dose by considering absorption, metabolism, distribution, and elimination. Levels III and IV include not only pharmacokinetic models, but also pharmacodynamic models, indicated in Figure 1 as "Toxicant-Target Models".

Mathematical models of the mechanistic determinants of the disposition of a parent compound and/or its metabolites, such as PBPK or dosimetry models, have been useful in describing the relationship between exposure concentration and target tissue dose. These disposition models can be linked to other models that address the mechanistic determinants of the toxicant-target tissue interaction and tissue response, respectively. These latter models refine the designation of response. The tissue dose is linked to determinants of target-tissue interaction (e.g., critical mechanistic events such as cytotoxicity and rebound cellular proliferation), which, in turn, may then be related via other mechanisms to the ultimate production of lesions or functional changes that are typically defined as the disease (pathogenesis) outcome. To the extent that these events are explanatory of the disease outcome, they can be used to quantitate important nonproportionalities or as replacement indices of the response function (U.S. EPA, 1994). It is important to emphasize that the integration of the mechanistic determinants may not necessarily be achieved by linking respective models in a series (.e., the output of one model becomes the input to the next) but may require simultaneous solution (e.g., the mechanistic determinants of disposition are dynamically related "moment-by-moment" to mechanisms of toxicant-target interaction). Eventually, causality of the critical mechanistic toxic effect can be correlated to the internal toxic moiety as the dose surrogate, rather than relating the exposure concentration to the "black box" of the organism within a population.

The characteristics of exposure which become important in understanding the exposure-dose-response continuum are not only the magnitude, duration and frequency of exposure, but also agent-specific characteristics such as the reactivity, solubility, and volatility. Char-

Figure 1: Comprehensive Exposure-Dose-Response Continuum

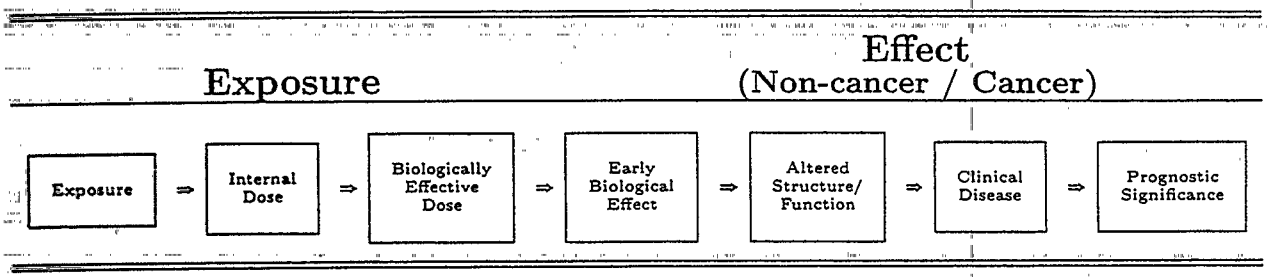


(Adapted from Jarabek, 1995)

acterizing attributes of the portal-of-entry (e.g., barrier capacity, cell types and morphology, specialized absorption sites, pH) and its interactions with the agent are important to defining dose, both for "local" target toxicity, as well as how it modulates systemic delivery. Systemic parameters that modulate target tissue dose include metabolism, clearance, tissue binding, blood flows, tissue volumes, and partition coefficients. An understanding of how the exposure is translated into a "dose" also depends on whether the important determinant of toxicity is the concentration of the parent compound or some metabolite, and the stability or binding of the parent compound or metabolite. Finally, the response depends on repair processes, cytotoxicity, cell proliferation, altered gene expression, and adaptation.

There is a striking similarity between the exposure-dose-response paradigm shown in Figure 1 with that proposed by molecular epidemiologists for considering the role of biological marker components in sequential progression between exposure and disease, as

Figure 2: Biological Marker Components in Sequential Progression Between Exposure and Disease (adapted from Schulte, 1989)



shown in Figure 2 (Schulte, 1989; US EPA, 1994). As defined by the National Research Council (NRC) Board on Environmental Studies and Toxicology, a "biologic marker" is any cellular or molecular indicator of toxic exposure, adverse health effects, or susceptibility. The markers may represent signals - - generally biochemical, molecular, genetic, immunologic, or physiologic - - in a continuum of events between a causal exposure and the resultant disease. It should be emphasized that the components shown in Figure 2 are not necessarily discrete events or the only events in the continuum; there may be a series of other components (steps or stages) between or in parallel with those shown that have yet to be discovered.

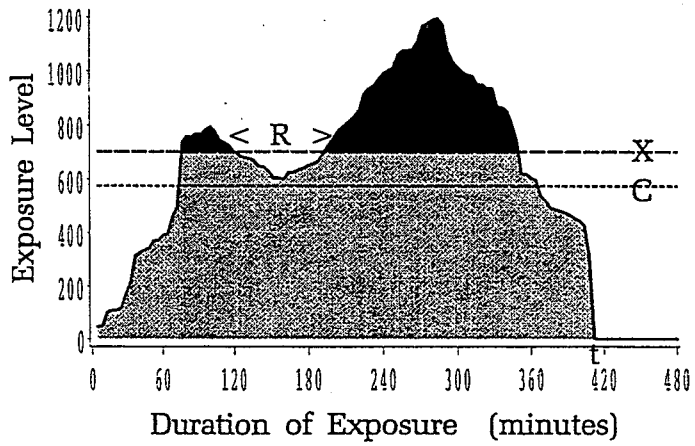
The molecular epidemiology approach is based on the combination of two biological tenets: (1) Early biologic effects from a toxic exposure are far more prevalent in the population at risk than the later events which have historically been of direct interest such as disease, and may sometimes be more specific to the exposure than the outcome itself; and (2) Given technological advances, most xenobiotics can either be directly quantified in the body or indirectly measured by identification of some predictable, dose-related biologic. Thus, the historic analytic epidemiology approach may be supplanted by a more in-depth approach which identifies intervening relationships more precisely or with greater detail than in the past. As a result, health events are less likely to be viewed as dichotomous phenomena (presence or absence of disease) but rather as a series of changes in a continuum from homeostatic adaptation, through dysfunction, to disease and death (Schulte, 1989).

The similarity of the dosimetry and molecular epidemiology paradigms emphasizes that quantitative consideration of the events in the exposure-dose-response continuum has implications for dose-response assessment; insights may be gained on (1) how to extrapolate from high to low exposure levels, (2) the reliability of extrapolation from laboratory species to humans, (3) the relevance of certain physiologic events to disease outcome, and (4) an index of human interindividual variation (U.S. EPA, 1994).

In the most general case, we can view the exposure profile as involving a continuous curve representing exposure levels over time. This is illustrated in Figure 3, adapted from Jarabek (1995). This figure suggests that there are many possible metrics for summarizing the effects of exposure on a toxic outcome. Some possible metrics are listed in Table 2. In some cases, more than one of the metrics might be required to fully characterize the exposure-response relationship. For example, Williams *et al.* (1996) found that the effects

Figure 3:

General Exposure Profile



C represents the mean concentration (1), t reflects the duration of exposure (2), X corresponds to a threshold level, and metric (5) is shown as the darkened area above X. R indicates the recovery period (7) between exceeding the threshold, and the AUC (4) is represented by the union of the gray and black solid areas.

Table 2: Dose Metrics Based on Exposure to Parent Compound or Metabolites

	Description
(1)	average concentration level, $\bar{C}$
(2)	duration of exposure, t
(3)	time-weighted average concentration, $\bar{C} \times t$
(4)	area under the curve, AUC
(5)	area over a certain threshold x
(6)	area under the curve when threshold x is exceeded
(7)	duration of recovery periods between exposures exceeding threshold x

of heatshock exposures on developing embryos were best modeled by including both the $C \times T$ metric of cumulative exposure and an additional effect for duration of exposure (i.e., metrics (2) and (3)), to account for the fact that shorter exposures at a higher level of heat stress resulted in greater morphological impairment than longer exposures yielding the same cumulative exposure. Both ozone and sulfur dioxide exposures show characteristic episodes of ambient-type exposures, i.e., increasing throughout the day to a peak and then decreasing. For ozone, short-term, high concentration exposures were identified by many researchers as being more important than long-term, low concentration exposures (see U.S. EPA, 1996a). Acknowledging this fact, Lefohn and Benedict (1982) introduced an exposure parameter that accumulated all hourly concentrations equal to and above a threshold level of 0.10 ppm, which corresponds to metric (5) in Table 2.

For exposure to chemical compounds, mechanistic properties of the chemical (deposition, absorption, distribution, metabolism, and elimination) lead to the question of whether

the important dose metric should be measured on the basis of the parent compound or an important metabolite, and whether they should be measured based on an external exposures or concentrations at some target tissue. The dose metrics defined in Table 2 could potentially refer to either the administered or ambient exposure level of the parent compound, a blood or tissue concentration of the parent chemical, or a blood or tissue concentration of an important metabolite. It should also be noted that the metrics listed in Table 2 are very generally described and would require additional specification for use in practice, such as the starting point in time after which exposures are measured.

Physiologically based pharmacokinetic (PBPK) models have been employed with some success to estimate levels of chemical disposition at target tissues. By appropriate scaling of mechanistic parameters, such as metabolic rates via the mixed function oxidase (MFO) or glutathione (GST) pathway, a more accurate estimate of target exposure levels in humans can be obtained. In some cases, it is likely that application of a PBPK model will provide an exposure metric which removes the need to subsequently account for duration of exposure through Haber's law or a more flexible dose-response model. Unfortunately, PBPK models have only been developed for a small minority of compounds of interest. In addition, estimates of mechanistic parameters like lipid partition coefficients obtained from other studies are treated as if they are "known", which results in underestimates of population variability.

While the exposure profile shown in Figure 3 attempts to illustrate the possible complexity of exposure profiles, controlled experiments rarely collect data at this level of detail. In situations where the exposures are controlled by the investigator, intermittent and highly variable exposure patterns are difficult to simulate and control, and the number of such patterns that would need to be considered would be impractical. Instead, investigators have considered a much more limited range of exposures, such as holding concentration steady over a certain duration and comparing to other constant $C \times T$ exposure blocks (see Figure 4). Another possible simplified exposure scenario which would represent repeated bursts of exposure relevant to many occupational settings could be to compare exposure patterns such as those illustrated in Figure 5. For example, hospital employees involved in sterilization of surgical instruments may be exposed to high concentrations of ethylene oxide over periods of a few minutes when opening hoods to load and unload equipment, and such exposures may occur repeatedly throughout the day. In other situations, investigators have not attempted to "control" exposure patterns, but have instead collected observational data by measuring both the exposure pattern over time and observed effects. These types of studies can give some sense of association between exposure patterns and toxic endpoints, but are not able to address causal hypotheses due to the limitations of observational studies.

In contrast to the complexity of possible exposure patterns which we might expect to observe in nature, the exposure scenarios exhibited in Figures 4-5 are simplified scenarios for controlled studies; they may not be ideal, but allow an initial assessment of dose-rate effects. The design of such studies will be discussed further in Section 4.2. Both in the design of studies and the modeling of the effects of exposure levels and duration on toxicity, there is often a gap between the underlying variability in exposure levels over time and the extent to which current methods allow modeling of such variability.

Figure 4:
Constant Blocks of Exposure

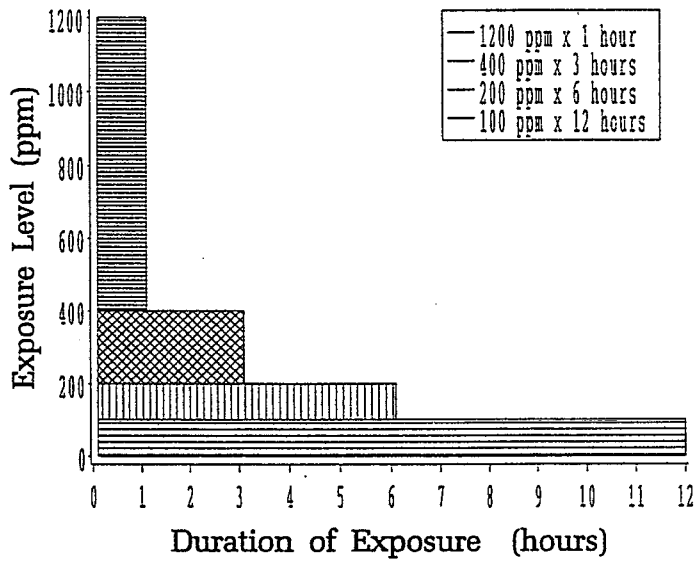
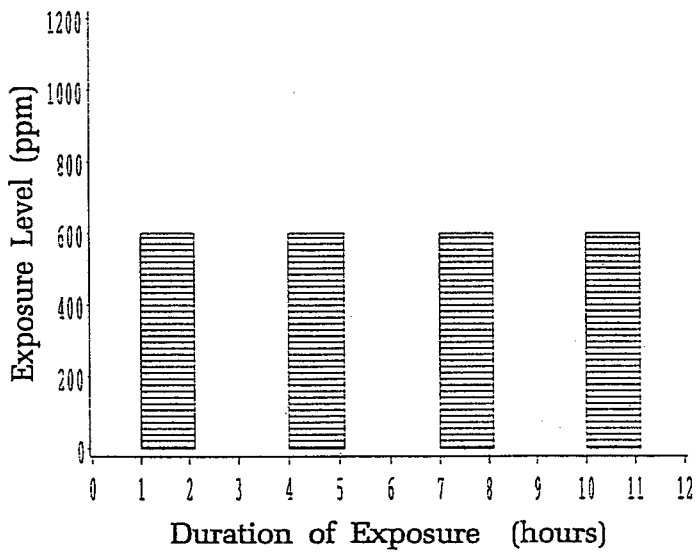


Figure 5:
Spiked Patterns of Exposure



An additional issue related to the dosimetry of exposure is the timing of exposure. The timing of exposure can affect the magnitude of toxic effects resulting from a given exposure due to periods of increased sensitivity. For example, for developmental toxicity outcomes, an exposure during the early part of the gestational cycle may result in effects of a much different type and severity than an exposure to the same agent and at the same level and duration but later in the gestational cycle (Daston and Manson, 1995). It is possible that the timing of exposure can be incorporated into the dosimetry of the exposure if the metric of interest is concentration of the parent compound or metabolite at a target organ, since the timing of exposure may impact the transport of the agent to the target tissue. However, in cases where less mechanistic information is known about the agent in question, the timing of exposure becomes an additional covariate that must be accounted for, leading to a multivariate vector of exposure covariates rather than a single summary metric.

4 Statistical Approaches for Assessment of Dose-rate Effects

4.1 Models for Dose-Rate Effects

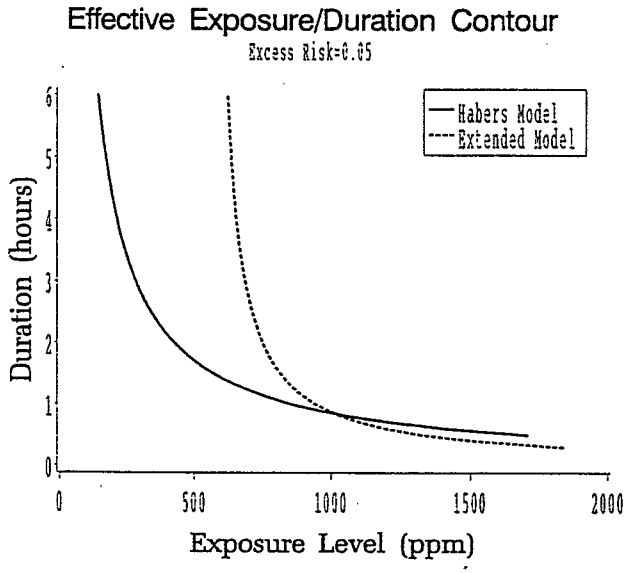
Many of the statistical approaches for dose-response modeling have dealt with exposures and toxicological effects only at the systemic level, treating "dose" as a black box, i.e., as in Level I of Figure 1. While development of more biologically-based models is certainly ideal, use of a dose-response model at this most superficial level can still be useful. In some cases, this effort can serve as a first step in attempting to identify whether non-linearities are apparent, and then attention can be turned towards identifying sources of non-linearities. Use of Haber's Law essentially implies treating the toxicological data at the systemic level, and statistical models may be able to examine the bounds under which Haber's Law holds for different substances. In other cases, however, it may be very difficult or impractical to consider dose-response modeling without additional mechanistic and kinetic models. The following section addresses the situation where systemic models of exposure-response relationships are felt to be of interest despite the fact that more biologically based mechanistic information may not be available.

According to Haber's Law, an effect level (EL) corresponding to a fixed incidence level or severity level for a given outcome is based on the following underlying relationship:

$$EL = a\, C^n \times t \tag{1}$$

where a and n are both empirically defined coefficients. The key implication of this model is that any pair of values (C, t) which yield the same multiple $(C^n \times t)$ would be assumed to produce the same level of toxic effect. This relationship can be summarized by an isobol of constant effect level, as shown in Figure 6 (model 1). For example, if the effect level of interest was the ED_{05} for fetal death resulting from a reproductive study and the exponent n equalled 1, then one could say that the ED_{05} level was proportional to the cumulative exposure. This implies that equivalent values of the ED_{05} might be a 6-hour exposure at

Figure 6:



300 ppm and a 9-hour exposure at 200 ppm. Based on 1-hour lethality studies, ten Berge *et al.* found that 19 of the 20 chemicals they evaluated had estimated values for n of 1-3.5. Thus, use of the relationship given by (1) assuming $n = 1$ for these 20 chemicals will tend to overestimate the exposure level corresponding to shorter durations, and underestimate the exposure level associated with the same effect level at longer durations.

These comments point to two important considerations. One is the need to account for chemical specific information on the *same endpoint* to establish an exposure-response relationship. Most of the 20 studies considered by ten Berge were lethality studies, and even if a given study yielded data which satisfied (1), this would be tenuous grounds for assuming that endpoints of milder severity such as the NOAEL would also follow this relationship. The other is that it is often tempting to use a relationship such as (1) to estimate the effect levels for durations of exposure that were not considered in the experimental design of the study conducted. Without a great deal of external mechanistic information, extrapolation of effect levels to durations of exposure not contained within the range of durations for a given set of studies is on very shaky ground. As noted by Bliss and James(1966), Haber's law tends to apply to either end of the exposure-response curve spectrum, i.e., at very high concentrations and short exposures or at low concentrations for longer durations, but not in between.

The relationship given by (1) above does not account for variability between animals or individuals in response levels. In order to estimate the probability of toxicity at combinations of exposure levels and durations, a dose-response model which reflects inter-individual variability must be fit to experimental data. Standard dose response models are a function of dose only and do not include a mechanism to account for various durations of exposure. Scharfstein and Williams (1994) extended dose-response models to account for duration and dose-rate effects as follows. First, under Haber's law the important metric is the cumulative

exposure, $C \times T$, so the dose-response model under Haber's law becomes:

$$p(C,T) = \mathcal{F}[\alpha + \beta(C \times T)] \tag{2}$$

where $p(C,T)$ is the probability of an adverse event at concentration C and duration of exposure T, and $\mathcal{F}$ is a cumulative distribution function, such as a logistic or probit link function. These models are fit using maximum likelihood methods, which yield parameter estimates for α and β along with estimates of their standard errors. To reflect departures from Haber's model, an extended model can be fit which includes an extra effect of duration of exposure in addition to the $C \times T$ metric:

$$p(C,T) = \mathcal{F}[\alpha + \gamma T + \beta(C \times T)] \tag{3}$$

In some instances, it may be desirable to modify this model so that duration of exposure has no effect at the control dose ($C = 0$), as follows:

$$p(C,T) = \mathcal{F}[\alpha + \gamma(\delta_C T) + \beta(C \times T)] \tag{4}$$

where $\delta_C = 0$ if $C = 0$ and $\delta_C = 1$ if $C > 0$. The suitability of this model depends on the length and conditions of the control exposure. For example, to evaluate morphological impairment from heat stress of less than one hour, submersion of embryos in a water bath at body temperature was expected to have the same effect regardless of duration. In contrast, based on an inhalation study of ethylene oxide (Weller *et al.*, 1998b), it was possible that exposure of pregnant mice to air for up to 8 hours could impact adverse effect levels such as fetal weight, since mice were deprived of food and water during the exposure period.

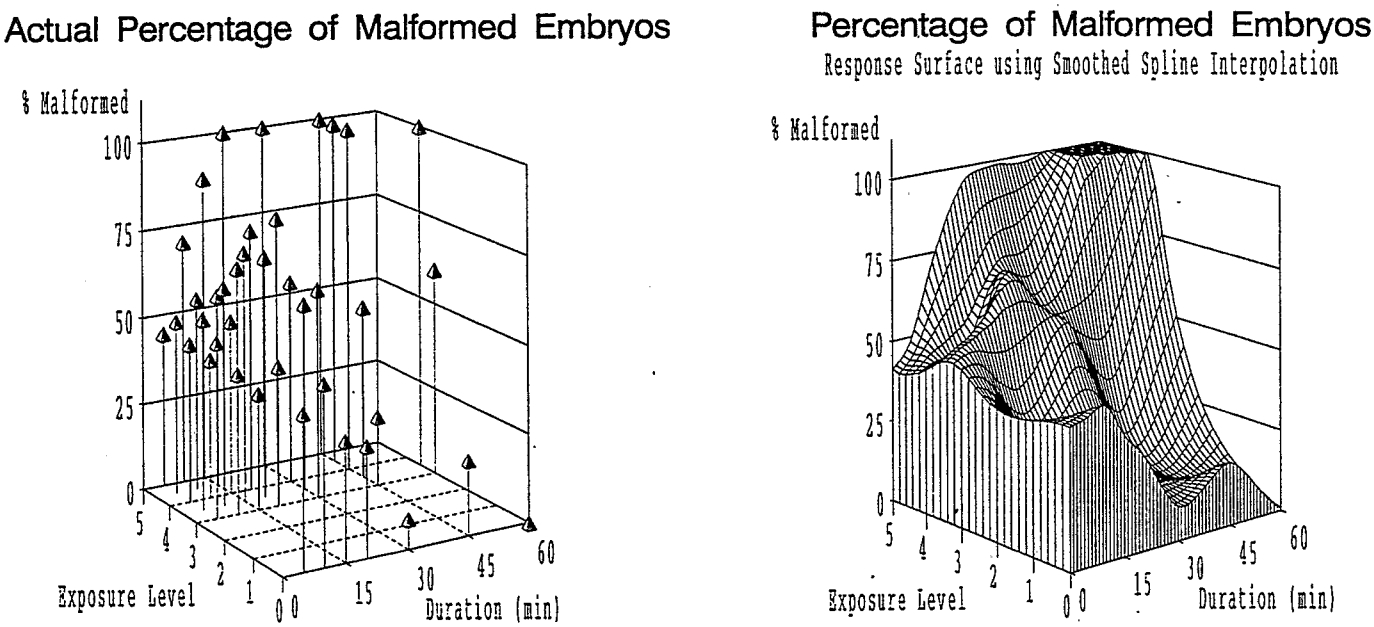
Based on fitting either of the extended models (3) or (4) above, one can conduct a test of whether Haber's model holds by testing whether $\gamma = 0$. Significant negative estimates of γ would imply higher toxicity rates for short high level exposures than for longer low level exposures given the same $C \times T$ multiple, while significant positive estimates of γ would imply the reverse. After fitting such models, one can also construct a "response surface", or the predicted probability of toxicity at each (C,T) combination (see Figure 7). The best fitting model can also form the basis for estimation of the effective dose-duration contour, i.e., the set of all (C,T) combinations that lead to an effect level of interest, such as the ED_{05}. This is an extension of the isobol for Haber's model shown in Figure 6.

Models such as (2) - (4) have been applied with some success to data resulting from heatshock studies (Williams *et al.*, 1996; Kimmel *et al.*, 1998, Geys *et al.*, 1998) and a dose-rate developmental toxicity study of ethylene oxide (Weller *et al.*, 1998b). In these contexts, the clustering of the animals into litters was accounted for by using generalized estimating equation (GEE) approaches. These models could also be easily extended to include a covariate for the timing of exposure. For example, in analysis of developmental toxicity data, one could include a covariate for gestation day (GD), as follows:

$$p(C,T) = \mathcal{F}[\alpha + \gamma T + \beta(C \times T) + \psi GD] \tag{5}$$

However, the amount of data that would be needed to fit such a model would be much larger than that for models (2)-(4), especially if there was interest in exploring interactions between gestation day and $C \times T$ effects.

Figure 7: Response Surfaces for Heatshock Data



The conceptual approach behind the models given in (2) - (4) can easily be extended to address both continuous and categorical outcomes, in addition to the binary toxicity outcomes considered above. For example, if the outcome of interest was fetal weight from a developmental toxicity study, a general model for estimating the mean fetal weight as a function of concentration and duration could be expressed as:

$$\mu(C,T) \;=\; \alpha + \gamma T + \beta(C \times T) \tag{6}$$

and under Haber's law γ would be set to 0. This type of model could be fit using generalized estimating equations to account for clustering, or by maximum likelihood in cases where data were not clustered. Such models are illustrated in the evaluation of fetal weight in the EtO study (Weller *et al.*, 1998b) and a set of six growth parameters, including crown rump length and yolk sac diameter, in the heatshock study (Kimmel *et al*, 1998). Modeling a continuous endpoint as shown above has the advantage of taking the actual measurements into account, and should thus be more informative in terms of identifying possible dose rate effects. However, approaches for risk assessment still typically depend on specifying a lower cutoff value (eg. for fetal weight, 2 standard deviations between the mean weight of control animals), below which animals are classified as "affected". This issue continues to be controversial in the assessment of continuous endpoints and will not be examined further here.

Similarly, the general model for binary outcomes given by (3) can be extended to ordinal outcomes, such as the severity of a malformation into 3 levels (normal, moderately affected, severely affected), by fitting an ordinal logistic regression model with covariates for concentration and duration:

$$Pr(Y \leq k|C,T) \;=\; \mathcal{F}\left[\alpha_k + \beta_1 T + \beta_2(C \times T)\right]$$

where Y reflects the severity level, and a separate intercept α_k is estimated for each severity level but a proportional odds assumption is made for the effect of exposure on the ordinal outcome. These models can be fit to single outcomes, and have also been extended to allow for joint modeling of multiple outcomes. These models have the advantage that they may provide for greater power in exploring dose-rate relationships.

A disadvantage of the models described above is that they treat the exposure data at the level of administered or external exposure only, and do not attempt to reflect the underlying mechanism of toxicity. Just as there is a shift in the area of cancer risk assessment to account for more mechanistic information in the risk assessment process, there has been a parallel shift in risk assessment for non-cancer outcomes. One such attempt is a generalization of Haber's law to account for detoxification:

$$D = [C\,V_m - De]\;tR/w \tag{7}$$

where D is the dosage received during time t, C is concentration, V_m is the respiration rate (m^3/min), De is the detoxification rate, w is body weight, and R is the retention coefficient. If the chemical is not detoxified at all ($De = 0$), then the relationship $D = aC \times t$ holds, with $a = V_m R/w$. However, if the detoxification rate is high, the dose received decreases. While this equation is a step in the right direction in terms of accounting for mechanistic information, most of the mechanistic parameters are unknown and not routinely estimated in toxicological studies.

4.2 Experimental Design

As noted previously, it is often impractical to design studies which evaluate the same types of highly variables exposures that are known to occur in the environment. Not only would the types of exposures shown in Figure 3 be difficult to control, but an evaluation of the effects of exposure as a function of such exposure patterns would require comparison with a seemingly unlimited set of exposure profiles. As a result, experimental designs for dose-rate studies, at least in the area of animal bioassays, have focused on exposure patterns such as those shown in Figure 4. There are again some exceptions to this general statement. For example, both Musselman et al. (1983) and Hogsett et al. (1985) developed experimental designs which allowed them to compare peak exposures to ozone versus mid-level exposures. Musselman et al. (1986) extended these earlier studies by further comparing exposure patterns with two different peak concentrations and two different exposure distribution curves (square wave and ambient wave).

While designs have been developed on an ad hoc basis to test the hypothesis of Haber's Law, there appears to be very little in the literature on *optimal* design of dose-rate studies. In other words, if the goal is to employ a whole animal-systemic approach and obtain the "best" characterization of the pattern of responses resulting from various dose-duration combinations, or have highest power for detecting departures from Haber's law, there is little guidance to draw from. Much of the work that has been done has focused on design of dose-rate studies for assessing developmental toxicity.

Using the modeling framework presented in the previous section, Scharfstein and

Williams (1994) consider the issue of optimal design for dose-rate studies. It should first be emphasized that the optimal design might depend on whether the goal of the study was to test for departures from Haber's model, or whether the objective was to obtain as accurate an estimate as possible of the combinations of (C, T) that lead to a certain effect level, such as the ED_{05}. In the first situation, the optimal design would be one which minimized the variance of the parameter γ, so that a test of $\gamma = 0$ would have high power. For the second objective, Scharfstein and Williams based comparisons of the efficiency of various designs on the distance between the "true" effective dose-duration contour, as based on a known dose-response model, and an "estimated" dose-duration contour based on Monte Carlo simulations of data under that true model and other possible models.

Initially, one might consider a design which investigated the combination of t duration levels $T_1, ..., T_t$ and c concentration levels $C_1, ..., C_c$, and assess the response level at all $t \times c$ combinations of duration and concentration. However, in many toxicological studies, it is likely that exposure for the highest duration T_t and concentration C_c would be associated with too high a level of mortality. In some cases, the range of exposures which lead to sufficient incidence rates for model fitting without excessive mortality can be fairly narrow. In addition, while this type of design might allow a better construction of the response surface, it would likely lead to a less efficient test of departure from Haber's law. Instead, it makes more sense to design studies which investigate several different $C \times T$ multiples and vary the duration of exposure within multiples. Comparison of toxicity rates within a given $C \times T$ multiple across the various durations of exposure thus gives a direct intuitive feel for whether Haber's law is met or violated.

Scharfstein and Williams considered the situation where 9 (C, T) combinations representing 3 multiples of $C \times T$ were to be evaluated, by specifying 3 different durations of exposure (1, 3, or 6 hours) at each multiple. The lowest multiple was assumed to apply to control animals ($C = 0$) and the highest multiple was fixed at 1200 ppm-hours in the context of an inhalation study, so that the issue of identifying optimal designs was reduced to the question of how best to choose the middle $C \times T$ multiple and how to allocate the animals to the 9 (C, T) combinations given a fixed number of pregnant dams. Within this framework, Scharfstein and Williams found that the optimal design for both of the objectives mentioned above tended to be the same. There were two designs which appeared to be robust to model departures and provide good efficiency for a wide range of dose-response relationships:

(1) equal allocation of animals to the 9 (C, T) groups, and specification of a middle multiple as 70% of the highest multiple, *or*

(2) allocation of twice as many animals to the 6 exposed groups as the 3 control groups, with specification of the middle multiple as 30% of the highest multiple

The first design described above was implemented in a study to investigate the dose-rate effects of ethylene oxide exposure on developmental toxicity. In this context and in many other types of toxicological studies, it seems essential to conduct a well-planned pilot study in preparation of the main study to investigate effects of timing. If resources prevent use of sufficient animals to include timing of exposure into the analysis based on a model such as (5), then the timing must be concentrated within a narrow enough range to isolate the

toxic endpoints of interest during that range. For example, in the EtO studies conducted at Harvard, we found that exposure during gestation day 6-8 yielded the most informative data on malformations of the type we were interested in, while exposures during earlier periods tended to result in greater fetal resorptions and fetal death.

Another complication of dose-rate studies which needs to be addressed in pilot the studies is the estimation of the MTD. In standard dose response studies, this would be the highest tolerated dose that does not lead to an increase in mortality or greater than 10%decrease in body weight. However, in dose-rate studies, one must identify the MTD taking duration of exposure into account. Our assumption in the EtO study was that short acute exposures would likely result in a higher rate of developmental toxicity, since the compound has a short half life and saturation is unlikely to occur. Consequently, we based our MTD on the shortest planned duration of 1 hour and identified the exposure level which resulted in an acceptably low rate of maternal toxicity. However, this short exposure duration was not associated with any fetal effects in our pilot study, so we subsequently extended the duration of exposure to 1.5 hours at a slightly higher exposure level. These experiences in designing dose-rate studies strongly suggest the use of pilot studies to identify the duration-dependent MTD and explore the issue of timing of exposure.

Thus far, the use of these study designs has optimistically designated a set of exposure durations and $C \times T$ multiples in advance, but in practice we may want to adapt the designs as a dose-rate study progresses to concentrate our resources at (C, T) combinations which will be most informative. The approach of continual reassessment methods (CRM) developed by O'Quigley and Pepe appears to be a promising framework for extension to such adaptive designs, but there may be other adaptive designs which should be considered in this context.

5 Future Directions

This issues paper has attempted to summarize the current approaches to conducting risk assessment in the context of dose-rate effects, and has raised a number of questions and issues that warrant further discussion and research.

Some general issues for discussion are provided below:

1. What biological level can or should be assessed in establishing a toxicologic exposure? Is it necessary to have an understanding of the mechanism of toxicity? How do such tissue and cellular functions as repair, metabolism, and bioaccumulation factor into defining the relationship? Is it possible to adequately describe a concentration duration relationship without measuring effects at biological levels below whole animal-systemic?

2. Many of the guidelines reflecting the endpoints in Table 1 are based on exposures of different durations than the guideline, and have used Haber's law for extrapolation purposes. Are these guidelines reliable? How can development of these guidelines be improved?

3. Are the models developed for assessing the joint effects of exposure levels and duration

reliable enough to allow extrapolation to doses not considered in fitting the models? How can these models be verified? How can we summarize uncertainty when extrapolating a model beyond the range of durations included in the data to which it was fit?

4. How can mechanistic information and exposure metrics derived from PBPK models be better incorporated into models which evaluate dose-rate effects?

5. How can study designs be improved to use information from previous exposures within the same or other studies to determine the most efficient subsequent exposure?

6. What is the appropriate metric to use for reflecting exposure levels and durations in dose-rate studies? How can the type of endpoint and the mechanism of action be utilized to help choose the appropriate metric?

7. What are the most important areas that require strengthening in the assessment of dose-rate effects?

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