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DRINKING WATER CRITERIA DOCUMENT FOR 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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The Office of Drinking Water (ODW), Environmental Protection Agency has prepared a Drinking Water Criteria Document on 2,4-D. This Criteria Document is an extensive review of the following topics:

- Physical chemical properties of 2,4-D
- Toxicokinetics and human exposure to 2,4-D
- Health Effects of 2,4-D in humans and animals
- Mechanisms of toxicological effects of 2,4-D
- Quantification of toxicological effects of 2,4-D

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1984; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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TABLE OF CONTENTS

		Page
I.	SUMMARY	I-1
II.	PHYSICAL AND CHEMICAL PROPERTIES	II-1
	DESCRIPTION	II-1
	PHYSICAL PROPERTIES	II-1
	SOLUBILITY IN ORGANIC SOLVENTS	II-1
	pK_a VALUES FOR 2,4-D	II-3
	VAPOR PRESSURES OF 2,4-D ESTERS	II-4
	ULTRAVIOLET ABSORPTION DATA FOR 2,4-D	II-4
	AQUEOUS DEGRADATION	II-4
	DIOXINS IN 2,4-D	II-7
	SUMMARY	II-7
III.	TOXICOKINETICS	III-1
	ABSORPTION	III-1
	DISTRIBUTION	III-3
	METABOLISM	III-8
	ELIMINATION	III-10
	SUMMARY	III-18
IV.	HUMAN EXPOSURE	IV-1
	To be provided by the Office of Drinking Water	
V.	HEALTH EFFECTS IN ANIMALS	V-1
	ACUTE TOXICITY	V-1
	SUBCHRONIC TOXICITY	V-4
	CHRONIC TOXICITY	V-13
	CARCINOGENICITY	V-15
	MUTAGENICITY	V-21
	TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS	V-33
	Teratogenicity	V-33
	Other Reproductive Effects	V-50
	SUMMARY	V-52
VI.	HEALTH EFFECTS IN HUMANS	VI-1
	ACUTE EFFECTS	VI-1
	SUBCHRONIC AND CHRONIC EFFECTS	VI-4
	Noncarcinogenic Effects	VI-4
	Carcinogenic Effects	VI-8
	SUMMARY	VI-15

TABLE OF CONTENTS (cont.)

		Page
VII.	MECHANISMS OF TOXICITY	VII-1
	SUMMARY	VII-6
VIII.	QUANTIFICATION OF TOXICOLOGICAL EFFECTS	VIII-1
	INTRODUCTION	VIII-1
	NONCARCINOGENIC EFFECTS	VIII-6
	QUANTIFICATION OF NONCARCINOGENIC EFFECTS	VIII-12
	Derivation of 1-Day HA	VIII-12
	Derivation of 10-Day HA	VIII-16
	Derivation of Longer-Term HA	VIII-22
	Assessment of Lifetime Exposure and Derivation of a DWEL	VIII-22
	Conclusions	VIII-27
	CARCINOGENIC EFFECTS	VIII-27
	QUANTIFICATION OF CARCINOGENIC EFFECTS	VIII-27
	EXISTING GUIDELINES, RECOMMENDATIONS AND STANDARDS	VIII-32
	SPECIAL GROUPS AT RISK	VIII-34
IX.	REFERENCES	IX-1

LIST OF TABLES

No.	Title	Page
II-1	Physical Properties of Some 2,4-D Compounds	II-2
II-2	Summary of Vapor Pressure Data for Various 2,4-D Esters	II-5
II-3	Conversion Factors for 2,4-D Esters	II-6
II-4	Dioxins in 2,4-D Samples	II-8
III-1	Plasma Half-Life Values of 2,4-D After Administration of a Single Oral Dose of 2,4-D Salt or Ester Equivalent to 100 mg 2,4-D/kg	III-11
V-1	Acute Toxicity of 2,4-D Compounds	V-2
V-2	Subchronic Toxicity of Orally Administered 2,4-D Compounds	V-7
V-3	Tumor Incidence in Rats Fed 2,4-D	V-19
V-4	Mutagenicity Testing of 2,4-D	V-22
V-5	Mutagenicity Testing of 2,4-D in Plants	V-25
V-6	Teratogenicity of Orally Administered 2,4-D and Derivatives of 2,4-D	V-34
VIII-1	Summary of Data Used to Derive HA and AADI	VIII-28

LIST OF ABBREVIATIONS

bw	Body weight
CNS	Central nervous system
CSF	Cerebral spinal fluid
DNA	Deoxyribonucleic acid
DWEL	Drinking water equivalent level
EEG	Electroencephalogram
FEL	Frank-effect level
GI	Gastrointestinal
HA	Health advisory
i.p.	Intraperitoneal
i.v.	Intravenous
LD_{50}	Dose lethal to 50% of recipients
LDH	Lactic dehydrogenase
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PCDD	Polychlorinated dibenzo-p-dioxins
ppb	Parts per billion
ppm	Part per million
ppt	Parts per trillion
RfD	Reference dose
RNA	Ribonucleic acid
s.c.	Subcutaneous
SGOT	Serum glutamic oxalacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
STEL	Short-term exposure limit

TLV	Threshold limit value
TWA	Time-weighted average
V_d	Volume of distribution

I. SUMMARY

2,4-Dichlorophenoxyacetic acid (2,4-D) (molecular weight, 221; water solubility, 540 mg/L at 20°C; pK_a, 2.87) is a white to yellow crystalline powder that is used as a herbicide for both terrestrial and aquatic plants and to prevent preharvest fruit drop in citrus trees, and also to increase the storage life of citrus fruit and increase the latex output of old rubber trees. 2,4-D has not been shown to contain 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), but does contain low concentrations (<60 ppb) of some other chlorinated dioxins. 2,4-D is degraded rapidly in water by chemical hydrolysis, photolysis and biological processes (the major removal mechanism).

Toxicokinetic studies have shown that most of the 2,4-D that is orally administered to animals (>90%) is excreted in the urine unchanged within 24-48 hours, suggesting fairly rapid and complete absorption and little metabolism of the compound. 2,4-D is widely distributed following absorption, but the highest concentrations are found in the liver, kidney, spleen, heart and lungs and the lowest levels in the muscle, brain and fat. Elimination from animals at low levels of exposure (<100 mg/kg) follows firstorder kinetics, but biphasic patterns are observed as concentrations increase. Limited human toxicokinetic data are consistent with the animal data, but considerable interindividual variations are noted in rates of absorption and elimination and the amount of the 2,4-D excreted as conjugates.

Acute exposure to 2,4-D by oral administration or injection by various routes results in progressive symptoms of muscular incoordination, hindquarter paralysis, stupor, coma and death in animals. Myotonia is a dominant effect of exposure, and lethal levels of 2,4-D have been shown to cause kidney and skeletal muscle damage in rodents. Oral LD_{50} values are generally in the range of 350-500 mg/kg bw for rodents; significant differences in toxicity are not apparent between 2,4-D and its salts and esters.

Subchronic oral administration of 2,4-D at daily doses of ~15.0 mg/kg/day caused alterations in hematology, in kidney and brain weights, and alterations in pituitary and adrenal weights, as well as in the liver enzymes LDH, SGOT, SGPT and alkaline phosphatase, which were biologically and stat- istically significant in mice and rats. Effects at 45 mg/kg/day or higher included GI disturbances as well as acute toxicity to hepatic tissues. Effects of higher doses included GI irritation and mild hepatic effects as well as symptoms and signs characteristic of acute exposures. Dogs appear to be more sensitive and guinea pigs less sensitive to subchronic oral administration of 2,4-D. Repeated s.c. or i.p. injection of 100-200 mg/kg bw 2,4-D cause pathological and functional effects in the liver, kidneys, lungs, thyroid, and nervous system of rats and mice, but systemic toxicity is not produced by daily dermal application of 2,4-D dimethylamine salt or esters to rabbits.

Chronic oral administration of 2,4-D at levels of up to ~78 mg/kg bw/day has no effect on hematological indices, clinical chemistry indices or non-tumor pathology in rats. Administration of 2,4-D in the diet of dogs for 2 years at levels up to ~14.5 mg/kg bw/day did not produce adverse gross or histopathological effects.

There are conflicting and unresolved reports of induction of lymphosarcoma in rats that were administered 2,4-D in the diet at levels in the range of 0.25-62.5 mg/kg bw/day for 2 years, but administration of 2,4-D or the isopropyl, butyl or isooctyl esters by intubation before weaning (46-100 mg/kg bw/day) and subsequently in the diet (~14-42 mg/kg bw/day) for 73-90 weeks was not tumorigenic. Rats or mice fed 2,4-D amine salt at 0.10% of the LD_{50} level for life reportedly did not develop a significant increase in tumors. Single s.c. injections of 2,4-D isooctyl ester were associated with reticulum cell carcinomas in mice after 78 weeks of latency, but similar injections of 2,4-D acid or the isopropyl or isobutyl esters were not tumorigenic. Repeated dermal applications of 2,4-D to mice produced skin papillomas only when treatment was preceded by application of the initiator 3-methylcholanthrene. A 1985 industry sponsored rat and mouse bioassay is available but on an interim basis has not been critically evaluated in this document. Note is made that EPA's Office of Pesticide Programs, in a March 23, 1988 Federal Register Notice propcses that the available animal evidence be viewed as inadequate to assess the carcinogenic potential in animals.

Five epidemiologic studies provide evidence of cancer induction from exposure to a class of compounds-chlorophenoxy herbicides. Both EPA and IARC have judged this evidence to be limited according to weight-of-evidence guidelines. The evidence for 2,4-D alone prior to a 1986 study by Hoar et al. was clearly inadequate, the Hoar et al. study raises questions as to whether the epidemiologic evidence for 2,4-D is now more substantial.

On an interim basis this document defers an evaluation of the human weight of evidence by the CAG in recognition of additional data forthcoming from NCI epidemiologic investigators. Note is made that the Office of Pesticide Programs (March 23, 1988) has proposed that the human data base including the Hoar et al., 1986 findings be considered inadequate for OPP regulatory use. The Pesticide Programs also propose that new animal studies be conducted and that the cancer evidence be reconsidered depending on the findings of such studies or the availability of newer epidemiologic information.

2,4-D has been tested for mutagenicity in a variety of assays (e.g., plant, bacteria, yeast, fruit flies, in vitro and in vivo mammalian systems), but there is a preponderance of negative and inconsistent results in the animal assays. It appears that these varied results may be attributed to differences in pH. At physiological pH, 2,4-D exists in the ionized state, where it less readily crosses cell membranes than when in the non-ionized state; the inconsistent results may indicate that sufficient levels of 2,4-D did not reach the target sites.

Teratogenicity testing with several species of rodents indicates that 2,4-D and several of its esters and other derivatives are embryotoxic, but only weakly teratogenic or non-teratogenic. Malformations generally consisted of cleft palate and other skeletal effects, but the threshold for adverse fetal effects is not clearly defined; sporadic evidence of mild fetotoxicity was reported in orally treated rats at doses as low as 12.5-25 mg 2,4-D/kg bw/day for both 2,4-D and 2,4-D esters. Multigeneration studies indicated that 2,4-D caused increased mortality in preweanling rats, but produced no adverse effects on litter size or fertility.

Reports of humans who acutely ingested 2,4-D solutions or were exposed to 2,4-D formulations via industrial or agricultural exposure indicate that symptoms of gastritis, vomiting, loss of consciousness, neurological signs (e.g., reflex disorders) and muscular paralysis precede death. Autopsies of fatal poisoning cases have shown widespread pathologic effects (e.g., congestion and hyperemia of most organs, hepatic necrosis). An inadequately reported epidemiology study concluded that chronic exposure to 2,4-D did not produce adverse clinical effects.

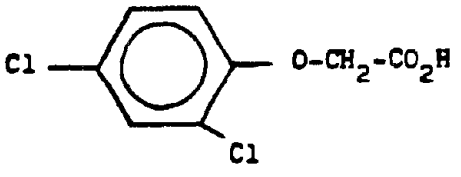
A non-lethal single oral dose in mice that represents a lowest-observed-adverse-effect level (LOAEL) was used to derive a 1-day HA for ingestion of 2,4-D in drinking water of 1.1 mg/ℓ for children. A 10-day HA of 0.30 mg/ℓ for children was derived from subchronic NOAELs in rats. A lifetime Drinking Water Equivalent Level (DWEL) of 0.35 mg/ℓ is recommended at this time based on a subchronic rat NOAEL.

II. PHYSICAL AND CHEMICAL PROPERTIES

Description

2,4-Dichlorophenoxyacetic acid (2,4-D) is a white to yellow crystalline powder that is used almost exclusively as an herbicide and to increase the latex output of old rubber trees, prevent preharvest drop in citrus trees and increase storage life of citrus fruit (Hawley, 1977; Ayers et al., 1976; Bovey and Young, 1980). It is also used as an herbicide for aquatic plants.

The chemical structure of 2,4-D, the Chemical Abstracts Service (CAS) Registry Number and the Registry of Toxic Effects of Chemical Substances (RTECS) number are given below.



CAS Number: 94-75-7

RTECS Number: AG6825000

Physical Properties

The physical properties of 2,4-D and four derivatives are presented in Table II-1.

Solubility in Organic Solvents (Herbicide Handbook, 1979)

acetone	-	850,000 mg/l at 25°C
ethanol (95%)	-	1,300,000 mg/l at 25°C
isopropanol	-	316,000 mg/l at 31°C
benzene	-	10,700 mg/l at 28°C

TABLE II-1

Physical Properties of Some 2,4-D Compounds[a]

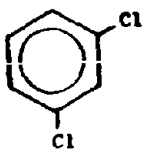
Compounds	Structure	Molecular Formula	Molecular Weight	Melting Point (°C)	Boiling Point (°C)	Density	Vapor Pressure (Torr)	Physical State	Water Solubility
2,4-D	$ArOCH_2COOH$	$C_8H_6Cl_2O_3$	221.04	140-141 135-138 (tech.)	160 (at 0.4 torr)	1.57 (at 30°C)	NA	white crystals, odorless when pure	540 mg/ℓ at 20°C (Melnikov, 1971); 725 mg/ℓ at 25°C (Bailey and Whit 1965); 900 mg/ℓ at 25°C (Herbici Handbook, 1979)
2,4-D Sodium Salt	$ArOCH_2COONa$	$C_8H_5Cl_2O_3Na$	243.03	216-218	NA	NA	NA	white crystals	27.5 g/100 g H_2O at 0°C: 33.5 g/100 g H_2O at 20°C: 50.6 g/100 g H_2O at 30°C
2,4-D Dimethyl-amine Salt	$ArOCH_2COONH_2(CH_3)_2$	$C_{10}H_{13}Cl_2NO_3$	266.12	85-87	NA	NA	10^{-9} at 20°C[b]	white odorless crystal-line solid	300 g/100 g H_2O at 20°C
2,4-D Butoxy-ethyl Ester	$ArOCH_2COOCH_2CH_2OCH_2CH_2CH_2CH_3$	$C_{14}H_{18}Cl_2O_4$	321.20	NA	156-162 (at 1-1.5 torr) 185-190 (at 5.5-7 torr)	NA	see Table II-2	viscous, colorless, odorless liquid when pure	12 mg/ℓ at 25°C
2,4-D n-Butyl Ester	$ArOCH_2COOCH_2CH_2CH_2CH_3$	$C_{12}H_{14}Cl_2O_3$	277.15	9	146-147 (at 1 torr)	1.235-1.245 at 25°C	see Table II-2	clear, colorless when pure	46 mg/ℓ at 25°C

[a]Sources: Weast, 1980; Herbicide Handbook, 1979; Melnikov, 1971; Zepp et al., 1975

[b]No reference or experimental method was provided for this value

NA = Not available

tech. = technical

Ar = 

toluene	-	6,700 mg/ℓ at 25°C	
xylene	-	5,800 mg/ℓ at 25°C	
diethyl ether	-	270,000 mg/ℓ at 25°C	
dioxane	-	785,000 mg/ℓ at 31°C	
n-heptane	-	1,100 mg/ℓ at 25°C	(Melnikov, 1971)
carbon tetrachloride	-	1,000 mg/ℓ at 25°C	
carbon disulfide	-	5,000 mg/ℓ at 29°C	

pK_a Values for 2,4-D

The pK_a values for 2,4-D in water at 25°C are listed below with the method of determination (2,4-D is a weakly acidic herbicide, pK_a is the negative log of the ionization constant):

pK_a	Method	Reference
2.87±0.6	spectrophotometric	Cessna and Grover, 1978
2.73	potentiometric	Nelson and Faust, 1969
2.96	potentiometric	Wedding et al., 1954
2.90	conductimetric	Matell and Lindenfors, 1957
2.92	conductimetric	Wershaw et al., 1967
3.28	unspecified	Andus, 1949

According to Cessna and Grover (1978), their spectrophotometric method yields the most accurate pK_a value claiming that potentiometric titration is not ideal for many herbicides because of their low solubilities; that the conductimetric method, although suitable at low concentrations, has to be performed at a number of dilutions, with each conductimetric value requiring different activity corrections; and that activity corrections are quite tedious. This results in the wide range of pK_a values (Cessna and Grover, 1978).

Vapor Pressures of 2,4-D Esters

Vapor pressures of several 2,4-D esters are listed in Table II-2. These data are not consistent, which is probably the result of the experimental method; however, even measurements using the same method can vary almost an order of magnitude. For example, 2,4-D isopropyl ester vapor pressure measured by gas liquid chromatography is reported to be 1.40×10^{-3} and 4.60×10^{-5} mm Hg at 25°C (see Table II-2). This variation makes calculations based on the vapor pressure difficult to interpret. Conversion factors for those esters listed in Table II-2 are presented in Table II-3.

Ultraviolet Absorption Data for 2,4-D

λ max = absorption wavelength, ε = extinction coefficient values

ionized		unionized	
λ max	ε	λ max	ε
290	1490	288	1310
283	1680	281	1500
229	7240	226	6960
202	26300	202	25500
in solution of 0.001 M KOH		in solution of 0.2 M HCl	

There is little difference in absorption spectra between the anionic and molecular forms (NRCC, 1978).

Aqueous Degradation

Chemical hydrolysis plays an important role in 2,4-D ester degradation in basic natural water but is minimal in neutral or acidic water (Zepp et al., 1975; Mill, 1980). Zepp et al. (1975) estimated that the hydrolytic half-life of the methyl, isopropyl, n-butyl, 1-octyl and isooctyl esters at

TABLE II-2

Summary of Vapor Pressure Data for Various 2,4-D Esters*

Ester	Vapor Pressure (mm Hg)	Temperature (C°)	Method	Reference
Methyl	1.55×10^{-3}	26.6	Boiling point determinations	Mullison and Hummer, 1949
	12.70×10^{-3}	25	Transpiration	Warren and Gillies, 1952
	2.30×10^{-3}	25	Gas liquid chromatography	Jensen and Schall, 1966
Ethyl	0.86×10^{-3}	26.6	Boiling point determinations	Mullison and Hummer, 1949
	11.50×10^{-3}	25	Transpiration	Warren and Gillies, 1952
	1.10×10^{-3}	25	Gas liquid chromatography	Jensen and Schall, 1966
n Propyl	7.20×10^{-3}	25	Transpiration	Warren and Gillies, 1952
Isopropyl	1.20×10^{-3}	26.6	Boiling point determinations	Mullison and Hummer, 1949
	10.50×10^{-3}	25	Transpiration	Warren and Gillies, 1952
	1.05×10^{-2}	25	Transpiration	Vernetti and Freed, 1963
	1.40×10^{-3}	25	Gas liquid chromatography	Jensen and Schall, 1966
	4.60×10^{-5}	25	Gas liquid chromatography	Flint et a , 1968
	2.32×10^{-4}	25	Knudson cell	Kybett et a.., 1976
n-Butyl	4.50×10^{-3}	25	Radioactive tracer	Warren and Gillies, 1952
	3.92×10^{-3}	25	Transpiration	Vernetti and Freed, 1963
	3.97×10^{-4}	25	Gas liquid chromatography	Jensen and Schall, 1966
	6.16×10^{-5}	25	Knudson cell	Kybett et al., 1976
n-Pentyl	3.00×10^{-3}	25	Transpiration	Warren and Gillies, 1952
n-Hexyl	2.10×10^{-3}	25	Transpiration	Warren and Gillies, 1952
n-Heptyl	1.35×10^{-3}	25	Radioactive tracer	Warren and Gillies, 1952
	0.02×10^{-3}	25	Gas liquid chromatography	Jensen and Schall, 1966
Dodecyl	0.40×10^{-3}	25	Transpiration	Warren and Gillies, 1952
2-Ethyl hexyl	2.00×10^{-6}	25	Gas liquid chromatography	Flint et al., 1968
Isooctyl	2.00×10^{-6}	25	Gas liquid chromatography	Flint et al., 1968
	7.06×10^{-6}	25	Knudson cell	Kybett et al., 1976
Butoxy ethyl	1.70×10^{-3}	25	Radioactive tracer	Warren and Gillies, 1952
	4.50×10^{-6}	25	Gas liquid chromatography	Flint et al., 1968
Propylene glycol butyl ether	3.00×10^{-6}	25	Gas liquid chromatography	Flint et al., 1968

*Source: NRCC, 1978

TABLE II-3

Conversion Factors for 2,4-D Esters

Ester	Conversion Factor
Methyl	1 ppm = 9.66 mg/m³ 1 mg/m³ = 0.104 ppm
Ethyl	1 ppm = 10.2 mg/m³ 1 mg/m³ = 0.0978 ppm
n-Propyl Isopropyl	1 ppm = 10.8 mg/m³ 1 mg/m³ = 0.0926 ppm
n-Butyl	1 ppm = 11.4 mg/m³ 1 mg/m³ = 0.0879 ppm
n-Pentyl	1 ppm = 11.95 mg/m³ 1 mg/m³ = 0.0837 ppm
n-Hexyl	1 ppm = 12.5 mg/m³ 1 mg/m³ = 0.0798 ppm
n-Heptyl	1 ppm = 13.1 mg/m³ 1 mg/m³ = 0.0763 ppm
Tridecyl	1 ppm = 16.5 mg/m³ 1 mg/m³ = 0.0605 ppm
2-Ethyl-hexyl Isooctyl	1 ppm = 13.7 mg/m³ 1 mg/m³ = 0.0731 ppm
Butoxy ethyl	1 ppm = 13.2 mg/m³ 1 mg/m³ = 0.0413 ppm
Propylene glycol butyl ether	1 ppm = 14.4 mg/m³ 1 mg/m³ = 0.0694 ppm

pH 9.0 and 6.0 were 1.1 hours and 44 days, 17.0 hours and 710 days, 5.2 hours and 220 days, 5.2 hours and 220 days and 37 hours and 1500 days, respectively. 2,4-D esters are photolytically degraded in water by >290 nm light, but limited data suggest that this rate is significantly slower than hydrolysis (Binkley and Oakes, 1974a,b; Zepp et al., 1975). Although abiotic degradation of 2,4-D may be rapid and is clearly a significant removal mechanism, 2,4-D appears to be degraded predominantly by biological processes (half-life of 1-2 weeks once biodegradation is detected) (Aly and Faust, 1964; Demarco et al., 1967; Boethling and Alexander, 1979).

Dioxins in 2,4-D

A number of studies have reported the presence of chlorinated dibenzodioxins in phenoxy herbicides including 2,4-D (Table II-4). In addition to these samples with positive contamination, Cochrane et al. (1980) analyzed 1 mixed butyl ester, 3 dimethylamine salts and 10 acid samples that contained no detectable dioxins. Thomas (1980) detected dichlorodibenzodioxins in 3 of 30 samples. Norstrom et al. (1979) analyzed five older 2,4-D samples and found no dioxins. There are few toxicology data available on these dioxins.

Summary

2,4-D is a crystalline powder used as an herbicide for both terrestrial and aquatic plants. It does not volatilize from water (although some of the esters are volatile) but is not persistent in water. Biodegradation is the major removal mechanism from water, although chemical hydrolysis of the esters is rapid at basic pH and photolysis may be significant. 2,4-D has not been shown to contain 2,3,7,8-TCDD but does contain low concentrations (<60 ppb) of some other chlorinated dioxins.

TABLE II-4

Dioxins in 2,4-D Samples

2,4-D Form	2,4-D Acid % (ppb)	Concentration of PCDDs					Country of Manufacture	Reference
		Di- (ppb)	Tri- (ppb)	1,3,6,9- or 1,3,6,8-Tetra- (ppb)	2,3,7,8-Tetra- (ppb)	Hexa- (ppm)		
Acid	44.6	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	41.3	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	41.7	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	37.9	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	42.44	316	490	132	ND	NA	Canada	Cochrane et al., 1980
	43.10	275	587	136	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	42.9	409	551	210	ND	NA	Canada	Cochrane et al., 1980
	42.9	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	42.55	140	230	96	ND	NA	Canada	Cochrane et al., 1980
	42.55	ND	58	54	ND	NA	Canada	Cochrane et al., 1980
	42.74	ND	584	278	ND	NA	Canada	Cochrane et al., 1980
	42.74	5	54	20	ND	NA	Canada	Cochrane et al., 1980
	49.59	33	533	208	ND	NA	Canada	Cochrane et al., 1980
	42.74	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	ND	ND	ND	ND	NA	Canada	Cochrane et al., 1980
	NR	NA	NA	ND[a]	ND[a]	5-10	USA	Woolson et al., 1972
	NR	52.9	ND	ND	ND	NA	USA	Thomas, 1980
	NR	25.4	ND	ND	ND	NA	USA	Thomas, 1980
Iso-octyl ester	65.4	NA[b]	346	226	ND	ND	Canada	Cochrane et al., 1982
	65.4	722	79	717	ND	ND	Canada	Cochrane et al., 1982
	50	200	632	1752	ND	ND	Canada	Cochrane et al., 1982
	65.1	104	639	315	ND	ND	Canada	Cochrane et al., 1982
	60	238	825	852	ND	ND	Canada	Cochrane et al., 1982
	70	109	929	486	ND	ND	Canada	Cochrane et al., 1982
	48.1	NA[b]	NA[b]	384	ND	ND	Canada	Cochrane et al., 1981
	42.9	474	450	8730	ND	ND	Canada	Cochrane et al., 1981

TABLE II-4 (cont.)

2,4-D form	2,4-D Acid % (ppb)	Concentration of PCDDs: Di- (ppb)	Tri- (ppb)	1,3,6,9- or 1,3,6,8-Tetra- (ppb)	2,3,7,8-Tetra- (ppb)	Hexa- (ppm)	Country of Manufacture	Reference
Iso-octyl ester	55.2	674	422	466	ND	ND	Canada	Cochrane et al., 1981
	55.0	110	385	127	ND	ND	Canada	Cochrane et al., 1981
	55.0	362	53	414	ND	ND	Canada	Cochrane et al., 1981
	52.36	NA[b]	346	226	ND	ND	Canada	Cochrane et al., 1981
	52.17	722	79	717	ND	ND	Canada	Cochrane et al., 1981
	46.55	200	632	1752	ND	ND	Canada	Cochrane et al., 1981
	NR	104	639	315	ND	ND	Canada	Cochrane et al., 1981
	45.46	238	825	852	ND	ND	Canada	Cochrane et al., 1981
	61.57	109	929	486	ND	ND	Canada	Cochrane et al., 1981
	54.3	151	102	120	ND	ND	Canada	Cochrane et al., 1981
Mixed butyl ester	68.3	815	273	219	ND	ND	Canada	Cochrane et al., 1981
	67.2	503	659	311	ND	ND	Canada	Cochrane et al., 1981
	69.5	295	371	314	ND	ND	Canada	Cochrane et al., 1981
	67.8	293	385	175	ND	ND	Canada	Cochrane et al., 1981
	NR	NA[b]	NA[b]	317	ND	ND	Canada	Cochrane et al., 1981
	47.26	NA	NA	NA	NA	NA	Canada	Cochrane et al., 1981
	67.68	151	102	120	ND	ND	Canada	Cochrane et al., 1981
	67.57	264	35	148	ND	ND	Canada	Cochrane et al., 1981
	NR	102	684	317	ND	ND	Canada	Cochrane et al., 1982
	50.0	ND	ND	ND	ND	ND	Canada	Cochrane et al., 1982
	NR	200	160	210	ND	ND	Canada	Cochrane et al., 1982
Dimethyl amine salt	50.0	ND	38	54	ND	ND	Canada	Cochrane et al., 1982
	50.0	ND	584	278	ND	ND	Canada	Cochrane et al., 1982
	50.0	5	54	20	ND	ND	Canada	Cochrane et al., 1982
	50.0	ND	ND	ND	ND	ND	Canada	Cochrane et al., 1982
	60.0	33	533	208	ND	ND	Canada	Cochrane et al., 1982
	50.0	ND	ND	ND	ND	ND	Canada	Cochrane et al., 1982
	50.0	ND	ND	ND	ND	ND	Canada	Cochrane et al., 1982
Propylene glycol butyl ester	48.8	134	418	384	ND	ND	Canada	Cochrane et al., 1982
Butoxy propyl ester	NR	59.7	ND	ND	ND	ND	USA	Thomas, 1980

[a]<0.5 ppm

[b]Not analyzed due to coeluting interferences

NA = Not analyzed; ND = Not detected at <1 ppb; NR = Not reported

III. TOXICOKINETICS

Absorption

Available data for nonruminant mammals indicate that absorption of 2,4-D from the GI tract is rapid and virtually complete.

Erne (1966a) administered various forms of 2,4-D by gavage to rats and pigs that were fasted overnight. When a single oral dose of 2,4-D triethanolamine salt equivalent to 100 mg/kg 2,4-D was given to male rats, peak plasma concentrations of 200 µg/mℓ 2,4-D were reached by 7 hours. Pigs that received 50 or 100 mg/kg of the compound had peak plasma levels of 120 and 210 µg/mℓ, respectively, by 5 hours postexposure. When rats were given single doses of the potassium-sodium salt of 2,4-D (equivalent to 100 mg/kg 2,4-D), absorption was similar to that of the 2,4-D amine. Similar administration of 2,4-D butyl ester (100 mg 2,4-D/kg) to rats resulted in much lower plasma levels (~20 µg/mℓ) than were reached at a later time (~7 hours). Erne (1966a) suggested that incomplete and delayed absorption of 2,4-D butyl ester accounted for the observed lower plasma levels, since its solubility in water is poor. Only the acid form of 2,4-D, rather than the intact ester, could be found in plasma at any time after dosing; thus, the author concluded that the ester underwent complete hydrolysis during absorption.

Khanna and Fang (1966) reported that 93-96% of an oral dose of 3-30 mg/kg of ^{14}C-2,4-D (acid) to rats was excreted almost entirely in the urine within 24 hours of dosing. Smith et al. (1980) recovered 90% of an administered dose in the urine of rats of up to 150 mg/kg 2,4-D as the sodium salt in 12 hours and 95% in 24 hours, indicating >95% absorption.

When ^{14}C-2,4-D was administered to rats as a bolus of 10 µmole/kg (containing 10 µCi/kg in a volume of 0.5 mℓ/kg) through the duodenal cannula, ~92% of the administered radioactivity was recovered in the urine after 24 hours (Sieber, 1976).

The rapid absorption of 2,4-D observed in experimental animals has been confirmed by studies that used human volunteers. Although individual variation was appreciable, significant levels of 2,4-D were detected in six men as early as 1 hour following ingestion of 5 mg/kg. Peak plasma levels were obtained within 7-24 hours and averaged ~35 µg/mℓ (Kohli et al., 1974; Khanna and Kohli, 1977).

In a similar study by Sauerhoff et al. (1977), oral administration of the same dose, 5 mg/kg 2,4-D, to three men also resulted in rapid absorption with peak levels of between 9 and 25 µg 2,4-D/mℓ plasma, achieved within 4 hours after ingestion. Values for absorption rate coefficients (k_a) were calculated using a nonlinear parameter estimation program and were reported as 0.165, 0.202 and 0.415 h^{-1} for the three subjects.

Dermal application of 4 µg/cm^2 of 2,4-D acid in acetone to the forearms of six men resulted in total excretion of 5.8% of the administered dose in the urine collected over 5 days. Measurable amounts were detected in the urine as early as 4 hours after exposure, indicating rapid percutaneous absorption (Feldman and Maibach, 1974).

An abstract of a study by Draper and Street (1982) reported dermal exposure to 2,4-D (1.2-18 mg of 2,4-D was washed from the hands of men engaged

in spray applications) resulted in maximum urinary excretion between 16 and 40 hours after the exposure. Inhalation exposure was minimal; further details were not reported.

Distribution

Erne (1966a) conducted a study on the distribution of 2,4-D as the triethanolamine salt using rats and pigs. The animals were given single oral doses of 2,4-D equivalent to 100 mg 2,4-D/kg and were killed by exsanquination. Tissues containing the highest levels of 2,4-D in both species at 6 hours were the liver, kidney, lungs and spleen. These levels declined after 24 hours. Brain levels of 2,4-D in the pigs were relatively low, ~5% of plasma levels, but could be increased by repeated administration of toxic doses. Only trace amounts of 2,4-D were found in fat. The butyl ester form of 2,4-D was distributed in a similar manner, but lower tissue levels were achieved. Apparent volumes of distribution (V_d) of different 2,4-D compounds (amine, potassium-sodium salt, butyl ester), estimated from elimination data plotted on a semi-logarithmic scale, ranged between 25 and 50% of the body weight, intermediate between extracellular body volume and total body water. These estimates suggested that some of the compound entered cells. The results of the in vitro study by Erne (1966a) support this concept by reporting ~10% of plasma levels of 2,4-D were found in blood cells.

Khanna and Fang (1966) reported tissue levels of 2,4-D in rats killed at various times between 1 and 41 hours after receiving 1 or 80 mg of ^{14}C 2,4-D by gavage. Distribution was widespread; radioactivity was detected in all of 12 tissues examined. Peak concentrations were reached in the tissues

between 6 and 8 hours at the low dose with no detectable radioactivity after 24 hours. At the high-dose level, peak concentrations were reached at 8 hours, remained fairly constant for 17 hours and then declined; however, radioactivity was still detected after 41 hours. The highest levels of radioactivity were found in blood, liver, kidney, heart, lungs and spleen, with lower levels in muscle and brain. At 17 hours after the high dose and at 1 hour after the low dose, the kidney level exceeded that of plasma. The intracellular distribution of 2,4-D was also investigated in kidney, liver, spleen, brain, heart and lungs. Higher levels of radioactivity were found in the soluble and nuclear fraction and relatively low levels were found in the mitochondrial and microsomal fractions.

The observations of low accumulations of 2,4-D in adipose tissue (Erne, 1966a; Khanna and Fang, 1966) and the low level of absorption by intestinal lymphatics observed by Sieber (1976) are not surprising. Sieber (1976) collected samples of perirenal or mesenteric fat from rats killed 24 hours after administration of a bolus dose of ^{14}C-2,4-D (10 μmoles/kg) through a duodenal cannula during infusion with physiological salt solution. Only 1.0±1.7% of the administered radioactivity was found in body fat (estimated as 12% of body weight). This finding was consistent with the low chloroform: buffer partition coefficient (0.007±0.002) for 2,4-D, indicating very low lipid solubility.

Some studies (Erne, 1966a; Khanna and Fang, 1966) report that relatively low levels of 2,4-D were found in the brain. Erne (1966a) found that brain levels of 2,4-D could be increased by increasing the dose. In support of this observation are the results of Elo and Ylitalo (1977, 1979), who demon-

strated that 2,4-D levels in the brain and CSF of rats could be greatly increased if the animals were pretreated with high (250 mg/kg) s.c. injections of 2,4-D sodium salt before i.v. administration of 8 μCi/kg ^{14}C-2,4-D with a specific activity of 0.9 μCi/mg. The measure of radioactivity in tissues as a percentage of plasma levels 3.5 and 4.5 hours after ^{14}C-2,4-D administration showed plasma levels reduced to 67%, while liver levels increased ~3.5 times, and testis, lung, heart and muscle levels increased ~2-fold when compared with levels in saline-pretreated controls. Pretreatment greatly increased the brain level by 6.5 times (2.3% in controls, 15% in pretreated rats) and the CSF level by 23.5 times (0.4% in control, 9.4% in pretreated rats).

The low levels of 2,4-D in brain tissue after a low dose is assumed to be due to the functioning of the blood-brain barrier. Based on experiments investigating 2-chloro-2-methyl phenoxyacetic acid (MCPA) binding to plasma protein in vitro, the authors suggested that if plasma protein binding sites are saturated by the high exposure levels, more unbound ^{14}C-2,4-D is free to be distributed to tissues (Elo and Ylitalo, 1979). The binding of 2,4-D to serum protein has been confirmed by in vitro studies (Mason, 1975; Haque et al., 1975; Kuhne et al., 1979; Fang and Lindstrom, 1980). The greater enhancement of brain and CSF accumulation of 2,4-D at high doses may be due to disruption (by high circulating levels of unbound 2,4-D) of the blood-brain barrier, with increased influx or decreased efflux or a combination of both processes (Elo and Ylitalo, 1979).

This hypothesis is supported by a study by Pritchard (1980), who demonstrated that ^{14}C-2,4-D was capable of being actively transported by rabbit choroid plexus preparations in vitro. The uptake of 2,4-D by the choroid plexus was shown to be energy dependent (inhibited by metabolic inhibitors), saturable, and specific for organic anions. 2,4-D was also shown to inhibit the transport of 5-hydroxy-3-indole-acetic acid, a metabolite of the neurotransmitter, serotonin. Since 2,4-D has been found in brain tissues, Pritchard (1980) suggested that 2,4-D can be accumulated in the brain in much the same way as the kidney accumulates this substance (see Elimination Sections). Intracellular binding before efflux may account for the observed brain levels of 2,4-D and for the CNS toxicity.

The distribution of 2,4-D in pregnant animals has also been studied. Lindquist and Ullberg (1971) subjected late stage pregnant mice (gestational day not specified) to whole body autoradiography following i.v. injection of ^{14}C-2,4-D (10 µCi = 0.05 mg/mouse) and killing at 5 minutes, 20 minutes, 1 hour, 4 hours and 24 hours. The autoradiograms showed that 2,4-D accumulated in the visceral yolk sac epithelium to a small degree, entered the fetus and was eliminated from all tissues within 24 hours.

The ability of 2,4-D to cross the placenta was also investigated by Fedorova and Belova (1974), who administered a single intragastric dose of 0.05 mg/kg ^{14}C-2,4-D to pregnant rats on the 19th day of gestation. The rats were killed 24 hours later and the levels of radioactivity present in the uterus, placenta, fetus and intrauterine fluid were 2.7, 3.5, 4.7 and 4.9% of the administered dose, respectively.

Erne (1966a) also found that 2,4-D crossed the placenta of a sow fed 500 ppm 2,4-D during the entire term of pregnancy. Tissue levels of 2,4-D found in dead piglets (10 of 15 died) were 35 µg/g of liver, 27 µg/kg of kidney and 30 µg/g of lung. Upon delivery, the level of 2,4-D in the placenta was 45 µg/g.

Several forensic investigations have determined levels of 2,4-D in human tissue samples obtained at autopsy from people who had ingested fatally high doses. Nielsen et al. (1965) determined the following tissue levels (in micrograms of 2,4-D per gram of tissue) in an apparent suicide victim (a 23-year-old man who ingested ~62 g of 2,4-D): muscle, 70; spleen, 134; liver, 183; blood, 669; kidney, 63; brain, 12.5; adipose tissue, 165. Analysis of tissue samples taken at autopsy from an elderly man who died 6 days after ingesting an unknown quantity of 2,4-D revealed the following tissue concentrations of 2,4-D (in µg/g tissue): blood, 57.6; brain, 93.4; kidney, 193.4; liver, 407.9; and muscle, 17.5 (Dudley and Thapar, 1972). Geldmacher et al. (1966) reported the following tissue levels of 2,4-D (µg/g tissue) in two fatalities: case one (33-year-old woman) - blood, 480; brain, 62; kidney and liver combined, 113; and case two (51-year-old woman) - blood, 25; brain 164; liver, 116; lungs, 88; and heart, 63. Levels of 2,4-D determined in the tissues of a female suicide victim (in µg/g tissue) were reported in an abstract (Coutselinis et al., 1977): liver, 21; spleen, 12; and kidney, 82.

Quantitative comparisons of the above information are difficult to make because of the unknown amounts of 2,4-D ingested and the uncertainty of the time of death after ingestion (the elderly man died 6 days after ingesting

2,4-D). There was also much variation between the victims with respect to relative blood to kidney or liver to kidney ratios. These studies do, however, confirm the findings in animal investigations that 2,4-D is widely distributed throughout the body tissues after oral administration of high doses.

Sauerhoff et al. (1977) calculated apparent V_d for three men who ingested 5 mg/kg 2,4-D. The changes in plasma levels with time indicated a one-compartment model for subjects 2 and 3, while a two-compartment model seemed more appropriate for the data of subject 1 (biphasic plasma elimination curve). The Vd values were 238 and 294 mL/kg for subjects 2 and 3, respectively. Vd_1 (for the central compartment) was 83 and Vd_2 (for the slow exchange compartment) was 218 mL/kg for subject 1. These small values indicated that 2,4-D was not widely distributed to tissues. Kohli et al. (1974) determined a Vd of ~100 mL/kg by averaging the data from six men who had ingested 5 mg/kg of 2,4-D, which is additional evidence of little distribution into the tissues of 2,4-D at low doses.

Metabolism

In studies to determine the extent of metabolism of 2,4-D, Erne (1966b) administered the amine salt of the compound to pigs, either in 3 oral doses of 50 mg/kg each, in 23 oral doses of 50 mg/kg each, or in the feed at 500 ppm for 5 months. The percent conjugation of 2,4-D in the urine of 6 pigs ranged from 0-18% conjugation as determined by differential acid hydrolysis. No correlation between the different exposure regimens and the amount of conjugation was evident. No significant amount of conjugation was detected in plasma. When 2,4-D butyl ester was administered orally to pigs (either 3

or 23 doses of 50 mg/kg each) or to rats (single dose of 100 mg/kg), only trace amounts of esterified compound could be detected in plasma, urine, red blood cells or liver, indicating complete hydrolysis of the ester linkage in vivo. The report did not state at what time after exposure these determinations were made.

Khanna and Fang (1966) found only unchanged 2,4-D in urine and tissue extracts from rats given oral doses of ~3-300 mg/kg ^{14}C-2,4-D when determined by paper chromatography. Countercurrent separation of urine and tissue extracts, however, indicated the presence of a very small amount of a metabolite of 2,4-D. This metabolite accounted for ~0.25% of the radioactivity in urine samples, 0.7% in the lung extracts and 6.1% in the liver. Paper chromatography of the metabolite using a 2-propanol-NH_4OH-H_2O solvent system gave an R_f of 0.67-0.69, compared with an R_f of 0.55-0.59 for 2,4-D. Further characterization was not performed.

Whole body extracts of mice prepared after an s.c. injection of 100 mg/kg 2,4-D, 2,4-D butyl ester or 2,4-D isooctyl ester in dimethylsulfoxide failed to show the presence of 2,4-dichlorophenol, suggesting that cleavage of the ether linkage is not a major metabolic pathway in animals (Zielinksi and Fishbein, 1967). Grunow and Boehme (1974) found primarily unchanged 2,4-D in the urine of rats administered 200 mg/kg of 2,4-D; ~3% of the administered dose was identified as the glycine and taurine conjugates of the compound.

Limited human data also indicate that 2,4-D does not undergo biotransformation to any great extent. Of five men who ingested 5 mg/kg of 2,4-D,

four excreted between 4.8 and 27.1% of the administered dose as conjugated 2,4-D. The rest of the 2,4-D excreted (82%) was detected as unchanged compound (Sauerhoff et al., 1977). Similarly, ~10% of the total 2,4-D excreted in the urine of a man who had ingested an herbicide containing 2,4-D and mecoprop [i.e., 2-(4-chloro-2-methylphenoxy)propanoic acid], as the amine salts, was in the form of acid-labile conjugates. The total dose was unknown but was sufficient to result in unconsciousness and myotonia (Park et al., 1977). Urine samples, collected from six men who ingested 5 mg 2,4-D/kg, were analyzed by gas chromatography and found to contain no metabolic products of the compound (Kohli et al., 1974).

Elimination

The elimination of 2,4-D (administered as the triethanolamine salt, the potassium-sodium salt, or the butyl ester) from the tissues of rats and pigs was studied by Erne (1966a) following single oral doses equivalent to 100 mg 2,4-D/kg. Blood samples were collected at 2-3 hour intervals for 12 hours after dosing and then less frequently for up to 50 hours, and then were analyzed for 2,4-D. Plasma half-life values (Table III-1) were calculated from semilogarithmic dose-elimination curves. The linearity of the terminal curves indicated first order elimination rates. As seen from Table III-1, the elimination of 2,4-D from the plasma in rats occurred at a slightly slower rate after administration of the butyl ester than after administration of the amine salt or potassium-sodium salt. Intact butyl ester was detected only in trace amounts in plasma; 2,4-D acid was the predominant form present in plasma (and tissues) after oral administration of the ester.

TABLE III-1

Plasma Half-Life Values of 2,4-D After Administration of a Single Oral Dose of 2,4-D Salt or Ester Equivalent to 100 mg 2,4-D/kg*

Administered Compound	Species	Plasma Half-life (hours)
2,4-D amine	Rat, male	2.9 ± 0.4
	Rat, female	3.3 ± 0.5
	Pig	12 ± 2
2,4-D K-Na salt	Rat, male	3.5 ± 0.5
2,4-D butyl ester	Rat, male	6 ± 1
	Pig	10 ± 0.8

*Source: Erne, 1966a

Erne (1966a) observed lower rates of elimination of 2,4-D from tissue (tissue half-life values for rats ranged between 5 and 10 hours, for pigs between 10 and 30 hours) than from plasma. Elimination of 2,4-D was essentially complete within 72 hours. Thus, 2,4-D did not accumulate in the tissues examined (Erne, 1966a).

Zielinski and Fishbein (1967) compared the differences in whole body elimination from mice given single s.c. injections of 100 mg/kg 2,4-D, 2,4-D butyl ester, or 2,4-D isooctyl ester in dimethylsulfoxide. The mice were killed various times after dosing, homogenized, and the extracts analyzed by gas chromatography for each compound. The whole body half-life values (assuming first order kinetics) were reported as 4.1 hours for 2,4-D, 1.1 hours for 2,4-D butyl ester and 3.4 hours for 2,4-D isooctyl ester. The esters did not appear to be hydrolyzed, as 2,4-D was not detected upon methylation of the whole mouse extracts. Differences between the results of this experiment and the data of Erne (1966a) may be attributable to route of administration and the use of dimethylsulfoxide as a vehicle by Zielinski and Fishbein (1967). Erne (1966a) administered the butyl ester of 2,4-D as an emulsion of the ester in petroleum solvent, with water.

Fedorova and Belova (1974) reported that, following oral administration of ^{14}C-2,4-D to rats at a level of 0.05 mg/kg, 92.1% of the administered dose was excreted in the urine within 3 days, while 6.1% of the radioactivity was detected in the feces in this time period. The study demonstrated that 2,4-D was excreted in the milk of nursing rats given a single oral dose of 100 mg/kg ^{14}C-2,4-D immediately after delivery. Radioactivity was

detected in the GI tract of pups killed up to 7 days after birth, with the maximum amount of 2,4-D excreted during the second or third day.

The excretion pattern of ^{14}C-1-2,4-D or ^{14}C-2-2,4-D in rats was studied by Khanna and Fang (1966). No radioactivity was found in the expired carbon dioxide of rats that were given oral doses of 3-300 mg/kg, although 93-96% and 94-98% of the radioactivity was detected in the urine and feces within 24 and 48 hours, respectively, after dosing with 3-30 mg/kg ^{14}C-2,4-D. Almost all of the 2,4-D had been excreted in the urine, with a small unspecified amount in the feces. As the dose of ^{14}C-2,4-D was increased (60-300 mg/kg), the percentage of radioactivity recovered in the urine and feces declined in a linear fashion; excretion was not complete even 144 hours after administration of 300 mg/kg.

Elimination of 2,4-D from tissues appeared to be complete within 24 hours after low doses were given (Khanna and Fang, 1966). When the dose was raised to ~240 mg/kg ^{14}C-2,4-D, radioactivity was still detected at 41 hours and the elimination appeared to be biphasic, with the second phase becoming apparent ~30 hours after dosing. The half-life values for the initial phase of elimination from blood, liver, kidney, heart, lungs and spleen were averaged and reported as 3.1 hours (range: 3.0-3.5 hours) as opposed to ~0.58 hours when the dose was 3 mg/kg. These values were crude estimates based upon levels of radioactivity determined from animals killed sequentially at various times after dosing.

Smith et al. (1980) also observed a dose-dependent biphasic urinary excretion pattern of 2,4-D in rats. A maximum urinary excretion rate of 7 mg/kg/hour was determined for rats given single oral doses of 10-150 mg/kg of the compound or injected i.v. with doses of 5 or 75 mg/kg of the 2,4-D sodium salt. Urinary excretion showed a biphasic pattern, with half-life values of 2 and 21 hours estimated for the fast phase and for the slow phase, respectively. Urinary excretion patterns became nonlinear at dose levels $\geq$100 mg/kg 2,4-D. The observations of Khanna and Fang (1966) and Smith et al., (1980) on delayed elimination of 2,4-D at high doses suggest a saturation mechanism.

Sauerhoff et al. (1977) studied the pharmacokinetic profile of 2,4-D administered orally to five human male volunteers. Following ingestion of 5 mg/kg of the compound, the subjects excreted an average of 82% of the dose as unchanged 2,4-D in the urine collected over 6 days (range: 48-97%). The average urinary half-life of 2,4-D was estimated to be 17.7 hours (range: 10.2-28.5 hours), using a one-compartment linear model for each individual set of data. Plasma clearance of the compound occurred by apparent first-order process with an average half-life of 11.6 hours; however, one of the three subjects investigated showed plasma clearance kinetics that suggested a two-compartment model rather than a one-compartment model. The researchers did not establish the factors that accounted for the wide range of calculated kinetic values; the authors suggested that changes in levels of protein binding and kidney function may be important. A delayed peak in urine excretion was also observed in the subject for whom a two-compartment model was indicated (on the basis of biphasic plasma elimination).

The elimination of 2,4-D was similarly studied by Kohli et al. (1974) in six men who also ingested 5 mg/kg of the compound. Gas chromatography analysis of urine samples detected 2,4-D as early as 2 hours after ingestion. By 4 days, an average of 77% of the administered dose of 2,4-D had been excreted as unchanged compound. The calculated half-life for plasma clearance was 33±3.1 hours, which represents the mean for the six subjects. This half-life value for plasma clearance is ~3 times longer than that calculated by Sauerhoff et al. (1977); however, there were wide individual variations and small numbers of subjects in the two studies. After i.v. injection of 1 μCi of ^{14}C-2,4-D as a solution of 1 Ci/mℓ in propylene glycol to six men, 100% of the administered dose was recovered in the urine after 5 days. The estimated half-life of elimination of 2,4-D administered by this route was 13 hours (Feldmann and Maibach, 1974).

Young and Haley (1977) analyzed data collected from a case in which a young woman had survived the ingestion of a mixture of 2,4-D and Dicamba and was being treated for the intoxication. These investigators made certain assumptions: that the initial dose, D_0, was 12.29 g [20.1% of 100 mℓ mixture or 20.1 g x 0.9 (10% was lost because of gastric lavage) x 0.6788 (67.88% of the 2,4-D salt is free acid)]; that by the time of lavage, all 2,4-D had been absorbed; and an assumed time zero blood concentration (C_0) of an arbitrary value higher than the first measured blood level was picked. This value, 1100 μg/mℓ, was used to estimate the V_d.

$$D_0/C_0 = V_d = \frac{12.29\ g}{1100\ \mu g/m\ell} = 11.2\ \ell$$

Thus the data collected over 219 minutes for blood and urinary levels of 2,4-D were analyzed for best fit using an E.A.I. Pacer 500 Hybrid computer, and a one-compartment model for 2,4-D with an "interactive urinary excretion

pathway" (k' blood-urine) to account for Dicamba elimination. First-order rate constants (hr^{-1}) for the 2,4-D best-fit model were $k_{blood\text{-}feces}$ = 0.010; $k_{blood\text{-}urine}$ = 0.002; $k'_{blood\text{-}urine}$ = 0.030; k_e = 0.012 (without $k'_{blood\text{-}urine}$), k_e = 0.042 (with $k'_{blood\text{-}urine}$). The $t_{1/2}$ was 16.7 hours when the interactive urinary excretion pathway was not shared with Dicamba and 59 hours when Dicamba used this pathway. When a "tissue compartment" for the 2,4-D model was included in the computer analysis, no better fit was obtained. Even though many assumptions were made in the analysis, the results supported the view that 2,4-D is rapidly absorbed following oral exposure, is not accumulated to any great extent in tissues, and is rapidly eliminated from the body.

In urine collected over 5 days after the application of 4 mg/cm² of 2,4-D in acetone to the forearms of six volunteers, 5.8% of the applied dose was recovered (Feldmann and Maibach, 1974). The discussion of absorption of 2,4-D suggests that the exposure to humans engaged in 2,4-D spraying in experiments simulating operations is mainly dermal. Draper and Street (1982) reported in an abstract that maximum elimination of 2,4-D in the urine of male ground spray applicators occurred 16-40 hours after termination of exposure. Taskav et al. (1982) reported a relatively long retention time for 2,4-D in some of 11 subjects who participated in spraying with the herbicide, since an average of 5.05 µg of 2,4-D was detected in 12-hour pooled urine samples 5 days after exposure. The results of this study were widely variable and very poorly reported.

The half-life for elimination of 2,4-D calculated from data collected from men engaged in agricultural spraying of 2,4-D ranged from 35-48 h

after a single exposure (Nash et al., 1982). One-time ground application of 2,4-D by 26 men resulted in mean 24-hour urinary excretion levels of 0.002 mg/kg for applicators, 0.003 mg/kg for mixer-loaders and 0.004 mg/kg for mixer-load applicators. Maximum mean 24-hour urinary excretion of 2,4-D by 17 men exposed intermittently during aerial spraying were 0.006 mg/kg for pilots and 0.02 mg/kg for mixer-loaders.

Blood and urine levels of 2,4-D have been determined in humans who were exposed to 2,4-D during spraying operations, but exposure occurred by both the inhalation and dermal routes. The limited data available suggest that exposure is mainly by the dermal route, but do not provide a basis for estimating the contribution of the dermal route to total absorption.

A poorly reported study by Taskav et al. (1982) reported mean serum levels of 106.63 ng/mL, ranging from trace amounts to 482 ng/mL, from blood collected immediately after spraying 6 gallons (3.8 lb acid equivalent/gal) of 2,4-D for 3 hours by 11 male volunteers. Exposures were determined from denim patches on the necks, chests and backs (dermal) and from air filter monitors (inhalation). Average amounts of residues on the denim patches were 131.45 µg/sq ft, while air filter residues averaged 43.1-60.1 ppt.

Plasma levels of 2,4-D in four men during a workweek of spraying a 2% emulsion of 2,4-D in kerosene ranged from undetectable amounts (<0.02 µg/mL) to 0.1-0.2 µg/mL. In this study, the exposures were intermittent and occurred by both dermal and inhalation routes (Kolmodin-Hedman and Erne, 1980).

In contrast, Lavy et al. (1982) detected very little, if any, 2,4-D in the urine of 18 men engaged in aerial spraying of the herbicide. Of 524 urine samples collected during the day of exposure and for 5 days afterward, only ~150 contained detectable amounts of 2,4-D. Those men with detectable levels were crew members who actually performed the spraying. Denim patches analyzed for dermal exposure and air filters analyzed for inhalation exposure contained very little 2,4-D, indicating minimal exposure by either route.

Summary

2,4-D triethanolamine and potassium-sodium salts are readily absorbed from the GI tract of nonruminant mammals and reach peak plasma concentrations as early as 7 hours in rats and 5 hours in pigs (Erne, 1966a). 2,4-D butyl ester is less completely absorbed and appears to be hydrolyzed to the free acid before absorption. That 2,4-D is rapidly and almost completely absorbed from the gut is suggested by reports of high urinary recoveries (90-96%) of intact 2,4-D within 24-48 hours in rats after oral administration (Khanna and Fang, 1966; Smith et al., 1980; Sieber, 1976). Studies with limited numbers of human volunteers have confirmed that 2,4-D is absorbed rapidly; significant levels have been detected in the plasma as early as 1 hour and peak plasma levels have been reached as early as 4-7 hours after ingestion (Kohli et al., 1974; Khanna and Kohli, 1977; Sauerhoff et al., 1977).

The presence of 2,4-D in the blood and urine of humans who were exposed to 2,4-D during spraying indicate that absorption can occur by dermal or respiratory routes or both (Kolmodin-Hedman and Erne, 1980; Taskav et al.,

1982; Lavy et al., 1982; Draper and Street, 1982). Rapid percutaneous absorption is indicated by the detection of 2,4-D in the blood as early as 4 hours after experimental dermal application (Feldmann and Maibach, 1974).

Animal studies indicate that distribution of absorbed 2,4-D is widespread and rapid, with peak tissue concentrations occurring as early as 6-8 hours after oral exposure (Erne, 1966a; Khanna and Fang, 1966). The highest concentrations are found in the liver, kidney, spleen, heart and lungs, and the lowest levels in the muscle, brain and fat. Distribution of 2,4-D to tissues other than those involved with excretion of the compound is enhanced relative to plasma levels at high doses. 2,4-D can cross the placenta of mice, rats and sows (Lindquist and Ullberg, 1971; Fedorova and Belova, 1974; Erne, 1966a), an observation that is notable in view of the ionization of 2,4-D (pK ~3) at plasma pH (Erne, 1966a). Forensic investigators in humans confirm the findings of animal studies that 2,4-D is widely distributed throughout the body tissues after oral administration of high doses (Nielsen et al., 1965; Geldmacher et al., 1966; Dudley and Thapar, 1972; Coutselinis et al., 1977).

2,4-D appears to be excreted essentially unchanged regardless of dose, route of administration or animal species (Erne, 1966b; Khanna and Fang, 1966). More sensitive detection techniques have, however, provided evidence of metabolism to 2,4-dichlorophenol (Zielinski and Fishbein, 1967) and of conjugation with amino acids (Grunow and Boehme, 1974). Limited data from humans also indicate that 2,4-D is not biotransformed to a large extent, although some conjugation may occur (Kohli et al., 1974; Sauerhoff et al., 1977).

Elimination of low levels (<100 mg/kg) of 2,4-D from the plasma, tissues and bodies of animals is rapid (generally complete in 24-72 hours) and follows first-order kinetics (Erne, 1966a). 2,4-D is excreted almost completely in the urine. As the concentration increases, biphasic patterns are observed, indicating that the saturation of some process takes place (Khanna and Fang, 1966; Smith et al., 1980). Toxicokinetic studies of humans who ingested 5 mg/kg of 2,4-D have estimated (using one-compartment assumptions) a urinary half-life of ~17.7 hours (Sauerhoff et al., 1977) and a plasma half-life of ~33 hours (Kohli et al., 1974). An elimination half-life of 35-48 hours was calculated from data collected from men engaged in agricultural spraying of 2,4-D (Nash et al., 1982).

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as 2,4-D from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to 2,4-D is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Detailed information concerning the occurrence of and exposure to 2,4-D in the environment is presented in another document entitled "Occurrence of Pesticides in Drinking Water, Food and Air" (Johnston et al., 1984). This chapter summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food and air.

In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for 2,4-D from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the Summary section of this chapter provides some insight into the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. Levels of 2,4-D in drinking water vary from one location to another. The highest level of 2,4-D monitored in the available studies was 50 µg/ℓ in Oregon (Elliott, 1979), below the Maximum Contaminant [illegible] (MCL) of 100 µg/ℓ. In the national studies, the highest level of 2,4-D was 1.1 µg/ℓ (Boland, 1981). However, levels of 2,4-D in drinking water typically appear to be lower than these levels. Analysis of the National Screening Program for Organics in Drinking Water (NSP) (Boland, 1981) and the Rural Water Survey (RWS) (U.S. EPA, 1984) suggests that median levels of 2,4-D in drinking water systems would be below 0.5 µg/ℓ, and possibly below 0.01 µg/ℓ, because only 1 of 117 systems sampled in the NSP contained a level of 2,4-D above 0.5 µg/ℓ, and none of 92 systems sampled in the RWS contained a level above 0.01 µg/ℓ. 2,4-D may not be present in drinking water in some areas. The available monitoring data are not sufficient to determine regional variations in exposure levels for 2,4-D.

The daily intake of 2,4-D from drinking water was estimated using the assumptions presented in Table IV-1 and the values presented above. The estimates in Table IV-1 indicate that the daily intake of 2,4-D from drinking water ranges from 0.0-1.4 µg/kg/day. However, the values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

A tolerance level for 2,4-D in potable water of 100 µg/ℓ (negligible residues) has been established from certain specific aquatic uses of 2,4-D (21 CFR 193.100, April 1, 1979).

Diet. Data are limited on the dietary intake of 2,4-D in the United States. Dietary exposure to 2,4-D appears to be low; there have been no findings of 2,4-D in FDA adult market basket surveys since 1973. The

TABLE IV-I

Estimated Intake of 2,4-D from Drinking Water*

Drinking Water Concentration (µg/ℓ)	Intake (µg/kg/day)
0.0	0.0
0.01	0.0003
0.5	0.014
1.1	0.031
50	1.4

*Assumptions: 70 kg adult male consuming 2 ℓ of water/day.

average total daily intakes of 2,4-D, based on detectable levels of 2,4-D in market basket studies performed between 1965 and 1973, were calculated to range from 0.0006-0.07 µg/kg/day (FDA, 1982).

Detectable residues of 2,4-D were found in the FY 76 market basket survey for toddlers. The average daily intake, based on the residue levels detected in the toddler diet in that year, was calculated to be 0.0058 µg/kg/day (FDA, 1980).

It is expected that dietary levels of 2,4-D vary somewhat with geographical location, with higher levels occurring in foods from areas near the sources of 2,4-D exposure. However, because of insufficient data, no estimates could be made of variations in intake by geographical region.

Tolerance levels for residues of 2,4-D in foods and in and on raw agricultural commodities are presented in Table IV-2.

Air. Data on levels of 2,4-D in ambient air are limited. A maximum level of 4 ng/m³ (0.004 µg/m³) of 2,4-D was reported from air monitoring studies of 16 cities (Grover et al., 1976). Using a range of air levels of 2,4-D of 0.0-0.004 µg/m³, the respiratory intake of 2,4-D was estimated. Assuming that a 70 kg adult male inhales 23 m³ of air/day (ICRP, 1975), a range of respiratory intake of 0.0-0.0013 µg/kg/day was estimated. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Summary

Data on the intake of 2,4-D from drinking water, food and ambient air are insufficient for use in determining which of the three sources is the major contributor to total intake. FAO/WHO and EPA have established acceptable daily intakes (ADIs) for 2,4-D of 300 and 125 µg/kg/day, respectively

TABLE IV-2

Tolerances for 2,4-D in Foods and In and On Raw Agricultural Commodities[a]

Commodity	Tolerance (µg/kg)
Food	
Barley, milled fractions (exc. flour)	2,000
Oats, milled fractions (exc. flour)	2,000
Rye, milled fractions (exc. flour)	2,000
Sugarcane, molasses	5,000
Wheat, milled fractions (exc. flour)	2,000
Raw agricultural commodity	
Apples	5,000
Asparagus	5,000
Avocados	100 N[c] 1,000[d]
Barley	
forage	20,000
grain	500
Blueberries	100
Cattle	
fat	200
kidney	2,000
meat byproducts (exc. kidney)	200
meat	200
Citrus fruits	100 N[c] 1,000[d]
including pre- and post-harvest	5,000
Corn	
fodder	20,000
forage	20,000
fresh, including sweet[b]	500
grain	500
Cottonseed	100 N[c] 1,000[d]
Cranberries	500

TABLE IV-2 (cont.)

Commodity	Tolerance (µg/kg)
Cucurbits	100 [NC] 1,000[d]
Eggs	50
Fish	1,000[d] 1,000[f]
Fruits	
pome	100 [NC] 1,000[d]
small	100 [NC] 1,000[d]
stone	100 [NC] 1,000[d]
Goats	
fat	200
kidney	2,000
meat byproducts (exc. kidney)	200
meat	200
Grain crops	100 [NC] 1,000[d]
Grapes	500
Grasses	
forage	100 [NC] 1,000[d]
hay	300,000
pasture	1,000,000
rangeland	1,000,000
Hogs	
fat	200
kidney	2,000
meat byproducts (exc. kidney)	200
meat	200
Hops	100 [NC] 1,000[d]

TABLE IV-2 (cont.)

Commodity	Tolerance (µg/kg)
Horses	
fat	200
kidney	2,000
meat byproducts (exc. kidney)	200
meat	200
Legumes, forage	100 N[c] 1,000[d]
Lemons, post-harvest	5,000
Milk	100
Nuts	100 N[c] 1,000[d]
Oats	
forage	20,000
grain	500
Pears	5,000
Potatoes	200
Poultry	50
Quinces	5,000
Rice	100
straw	20,000
Rye	
forage	20,000
grain	500
Sheep	
fat	200
kidney	2,000
meat byproducts (exc. kidney)	200
meat	200
Shellfish	1,000[d]
Sorghum	500
fodder	20,000
forage	20,000

TABLE IV-2 (cont.)

Commodity	Tolerance (µg/kg)
Strawberries	50
	100 N[c]
	1,000[d]
Sugarcane	2,000
forage	20,000
Vegetables	
fruiting	100 N[c]
	1,000[d]
leafy	100 N[c]
	1,000[d]
root crop	100 N[c]
	1,000[d]
seed and pod	100 N[c]
	1,000[d]
Wheat	
forage	20,000
grain	500

[a]Sources: 40 CFR 180.142, July 1, 1981; 21 CFR 193.100, April 1, 1979.

[b]Kernel plus cob with husks removed

[c]From application to irrigation ditch banks in the Western United States

[d]From application to control water hyacinth

[e]For 2,4-D and/or its metabolite 2,4-dichlorophenol

[f]From application for Eurasian water milfoil control in dams and reservoirs of the TVA system

N = Negligible residues

(FDA, 1982). In addition, EPA has reported a maximum safe level of 2,4-D (from all sources) of 16 µg/kg/day (U.S. EPA, 1976). The intake of 2,4-D from drinking water, food and air appears to be below these levels.

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V. HEALTH EFFECTS IN ANIMALS

Acute Toxicity

Acute toxic levels of 2,4-D and certain of its salts and esters by different routes of administration are summarized in Table V-1. The LD_{50} range is generally between 300 and 1000 mg/kg; there do not appear to be significant differences in toxicity between the free acid and the various salt and ester derivatives. Hill and Carlisle (1947) determined oral LD_{50}s of 666, 375, 800 and 1000 for 2,4-D sodium salt in rats, mice, rabbits and guinea pigs, respectively; the maximum doses in these species not causing death were 333, 125, 200 and 333 mg/kg, respectively. Individual monkeys that were fed single doses of ~286 or 428 mg/kg of 2,4-D sodium salt or 286 mg/kg of 2,4-D ammonium salt regurgitated a large portion of the material, precluding determinations of lethal doses (Hill and Carlisle, 1947). Symptoms other than nausea were not described in these monkeys. Approximately 214 mg/kg of 2,4-D sodium salt was fed to another monkey without development of vomiting or "serious illness" (Hill and Carlisle, 1947). Comparison of the species sensitivity to 2,4-D indicates that dogs may show greater sensitivity to this compound (Rowe and Hymas, 1954). This greater sensitivity observed in dogs may reflect an inability of kidney processes in this species to effectively clear phenoxyacetic acids (Seiler, 1978).

Hill and Carlisle (1947) noted that fatal poisoning of several types of laboratory animals (mice, rats, guinea pigs, rabbits and monkeys) with the sodium and ammonium salts of 2,4-D produced similar symptoms. Animals died within several hours to 3 days following oral or i.p. administration of the salts. Progressive symptoms included muscular incoordination, lethargy, paralysis of the hindquarters, stupor, coma and death. The authors noted

TABLE V-1

Acute Toxicity of 2,4-D Compounds

2,4-D Form	Vehicle	Route	Species	Dose (mg/kg)	Response	Reference
Acid	olive oil	oral	mice	368	LD_{50}	Rowe and Hymas, 1954
Acid	olive oil	oral	guinea pigs	469	LD_{50}	Rowe and Hymas, 1954
Acid	olive oil	oral	rats	375	LD_{50}	Rowe and Hymas, 1954
Acid	gelatin	oral	rats	666 333	3/4 deaths 0/4 deaths	Hill and Carlisle, 1947
Acid	capsule	oral	dogs	100	LD_{50}	Drill and Hiratzka, 1953
Sodium salt	saline	oral	mice	375 125	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	oral	guinea pigs	1000 333	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	oral	rabbits	800 200	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	oral	rats	666 333	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	H_2O	oral	rats	805	LD_{50}	Rowe and Hymas, 1954
Sodium salt	H_2O	oral	guinea pigs	551	LD_{50}	Rowe and Hymas, 1954
Sodium salt	saline	i.p.	mice	375 125	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	i.p.	guinea pigs	666 333	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	i.p.	rats	666 25	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	i.p.	rabbits	400 200	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	i.v.	rabbits	400 200	LD_{50} tolerated dose*	Hill and Carlisle, 1947
Sodium salt	saline	s.c.	mice	280	LD_{50}	Bucher, 1946

TABLE V-1 (cont.)

2,4-D Form	Vehicle	Route	Species	Dose (mg/kg)	Response	Reference
Ammonium salt	saline	oral	rats	333	0/6 deaths	Hill and Carlisle, 1947
Ammonium salt	saline	oral	guinea pigs	33	0/6 deaths	Hill and Carlisle, 1947
Ammonium salt	saline	oral	rabbits	200	0/4 deaths	Hill and Carlisle, 1947
Isopropyl ester	olive oil	oral	mice	541	LD_{50}	Rowe and Hymas, 1954
Isopropyl ester	olive oil	oral	rats	700	LD_{50}	Rowe and Hymas, 1954
Isopropyl ester	olive oil	oral	guinea pigs	550	LD_{50}	Rowe and Hymas, 1954
Butyl esters	corn oil	oral	mice	713	LD_{50}	Rowe and Hymas, 1954
Butyl esters	corn oil	oral	guinea pigs	848	LD_{50}	Rowe and Hymas, 1954
Butyl esters	corn oil	oral	rabbits	424	LD_{50}	Rowe and Hymas, 1954
Butyl esters	corn oil	oral	rats	620	LD_{50}	Rowe and Hymas, 1954
Butyl esters	NR	oral	cats	820	LD_{50}	Konstantinova, 1970
Butyl esters	NR	oral	rats	920	LD_{50}	Konstantinova, 1970
Butyl esters	NR	oral	mice	380	LD_{50}	Konstantinova, 1970
PGBE esters	corn oil	oral	rats	570	LD_{50}	Rowe and Hymas, 1954
Crotyl ester	NR	oral	mice	580	LD_{50}	Fetisov, 1966
Crotyl ester	NR	oral	rats	452	LD_{50}	Fetisov, 1966

*Tolerated dose - largest amount causing no deaths

NR - Not reported; PGBE - propylene glycol butyl ether

that the skeletal muscle changes resembled those observed in congenital myotonia. Pathological examination showed cloudy swelling and enlargement of the kidneys in all species; liver damage (centrilobular degeneration and parenchymal damage) was noted only in dogs succumbing to massive doses of 2,4-D.

Drill and Hiratzka (1953) described myotonia with pathologic changes of GI mucosa irritation, moderate hepatic necrosis and mild renal tubular degeneration in dogs that were lethally poisoned by acute oral administration of 2,4-D at doses of 100-400 mg/kg.

Bucher (1946) found that myotonia persisted for 8-24 hours in strain A mice that were injected i.p. with sublethal doses (100-200 mg/kg) of 2,4-D. No significant differences were found in the effects produced when 2,4-D was administered s.c., i.p. or i.v.

Subchronic Toxicity

In a subchronic exposure feeding study, 200 B6C3F$_1$ mice were sorted into five groups of 40 animals, 20 of each sex per group, and fed the diet chow mixed with 97.5% pure 2,4-D (Hazelton Laboratories, 1983). Mice were fed 0.0 (controls), 5.0, 15.0, 45.0 or 90.0 mg/kg/day (calculated doses) of the diet-chemical mixture for 91 days. Criteria used to determine toxicity were survival, daily exam for clinical symptomology, weekly changes in body weights, growth rates, food intake, ophthalmological alterations, organ weight changes, and clinical, gross and histopathological alterations.

The results of the study demonstrated increases in mean white blood cell counts (both sexes) at 15.0 mg/kg/day, and increased platelet and reticulocyte counts (females only) at the 15-45 mg/kg/day dose levels. There were also statistically significant reductions in absolute brain weights (males only) at the 15.0 mg/kg/day or higher dose. Alteration of relative brain-to-body weight was found (females only) at the 15.0 mg/kg/day or higher groups. Statistically significant reductions in kidney weights (absolute and relative) were found in males (45.0 mg/kg/day or higher) and in females at the 90.0 mg/kg/day dose level. Statistically significant reductions in liver weights were found only in the 90.0 mg/kg/day groups of males. Pituitary glands were hypertrophied (statistically significant) (absolute and relative weights) in both sexes at 15.0 or 90.0 mg/kg/day groups. Adrenal glands were also increased (statistically significant) at the 15.0 mg/kg/day dose in males and at the 5.0 mg/kg/day dose in females. Ovarian weights were reduced (statistically significant) in the 15.0 mg/kg/day dose level. One control female died at week 12 and one female in the high-dose group died at week 1. These deaths were not treatment-related.

Hematological, hepatic and renal toxicity were demonstrated in a sister study in Fischer rats (strain #344) during a subchronic exposure feeding study performed at the Hazelton Laboratories (1983). 2,4-D (97.5% pure) was added to the diet chow and fed to the rats for 91 days at doses calculated to be 0.0 (controls), 1.0, 5.0, 15.0 or 45.0 mg/kg/day. In each of the five groups were 20 animals of each sex, 40 animals per treatment group or a total of 200 animals. Criteria examined to determine toxicity were survival, daily exam for clinical symptomology, weekly change in body weights, growth rates, food intake, ophthalmological changes, changes in organ weights, and clinical, gross and histopathological alterations.

The results of the study demonstrated statistically significantly reductions in mean hemoglobin (both sexes), mean hematocrit and red blood cell levels (both sexes), mean reticulocyte counts (both sexes), mean platelet counts (females only) and mean leucocyte levels (males only) at the 5.0 mg/kg/day dose or higher after 7 weeks. There were also statistically significant reductions in liver enzymes LDH, SGOT, SGPT and alkaline phosphatase at week 14 in animals treated at the 15.0 mg/kg/day or higher doses. Kidney weights (absolute and relative) showed statistically significant increases in all animals at the 15.0 mg/kg/day dose or higher at the end of the experimental protocol. Histopathological examination correlated well with kidney organ weight changes showing cortical and subcortical pathology. Increases in ovarian weights, T-4 levels and a decrease in BUN were reported but not considered to be treatment related.

Rowe and Hymas (1954) administered doses of 0, 30, 100 or 300 mg/kg/day 2,4-D to groups of 5 or 6 female rats (strain not specified) by intubation 5 times/week for 4 weeks (Table V-2). The 2,4-D was administered in olive oil that was emulsified in 5-10% aqueous gum arabic, and the controls were vehicle treated. Rats that received 30 mg/kg/day or less reportedly showed no adverse treatment-related clinical or pathological effects; but treatment with 100 mg/kg/day elicited GI irritation, depressed growth rate and slight cloudy swelling of the liver. Rats that received 300 mg/kg/day 2,4-D succumbed rapidly (additional details were not given) and died; severe GI irritation was reportedly the principal adverse effect observed.

TABLE V-2

Subchronic Toxicity of Orally Administered 2,4-D Compounds

Species/ Strain	Sex/No.	Vehicle	Purity	Dosage/Exposure	Dose[a] (mg/kg/day)	Response	Reference
Mice/B6C3F$_1$	20 each sex per group	diet chow	97.5%	NS	5.0, 15, 45 or 90	Significant reduction in brain weight and increased white blood cell counts at 15 mg/kg bw/day or higher; significant reductions in liver weights at high doses; pituitary gland hypertrophy at 15 mg/kg bw/day or higher; other effects also noted.	Hazelton Laboratories, 1983
Rats/Fischer 344	20 each sex per group	diet chow	97.5%	NS	1.0, 5.0, 15 or 45	Significant reductions in measured blood parameters at 5.0 mg/kg bw/day or higher; liver enzyme activities were reduced and increased kidney weights occurred with corresponding histopathology at 15 mg/kg bw/day or higher.	Hazelton Laboratories, 1983
Rat/NS young adult	5-6 f/group	olive oil/ gum arabic	NS	3, 30, 100 or 300 mg/kg/day by gavage 5 days/week for 4 weeks	2.14, 21.4, 71.4, 214	2.14 or 21.4 mg/kg had no adverse effects as judged by growth, behavior, mortality, hematologic and BUN values, organ weights, and gross and microscopic appearance of tissues; 71.4 mg/kg caused GI irritation, depressed growth, slight cloudy swelling in liver; 214 mg/kg caused rapid deterioration and death; GI irritation	Rowe and Hymas, 1954
Rats/ Long-Evans	No. ambiguous: ~8 treated or control M/ treatment period	diet	analytical standard grade	2000 ppm in diet x 4, 5 or 7 weeks	200	No effect on food consumption, no overt signs of toxicity, slight increase in amount glycogen/liver, slight decrease in amount RNA/liver, slight decrease in absolute and relative liver weights	Chang et al., 1974

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Vehicle	Purity	Dosage/Exposure	Dose[a] (mg/kg/day)	Response	Reference
Rats/NS young adult	5 F/group	diet	NS	100, 300, 1000, 3000 or 5000 ppm in diet x 113 days	10, 30, 100, NA, NA	No effect at 2 lowest doses as judged by mortality, growth, food consumption, hematologic and BUN values, organ weights, gross and microscopic appearance of tissues; 100 mg/kg produced "excessive" mortality, decreased growth, slight cloudy swelling in liver, food consumption NS at this dose; at 2 highest dosages, rats refused to eat	Rowe and Hymas, 1954
Rats/NS young	7 M/group	diet	purified commercial	0, 200 or 400 ppm in diet x 31 days, 100 ppm x 21 days, then 1000 ppm for the subsequent 10 days (390 ppm TWA)	0, 10, 20 or 40 39[b]	No adverse effects as judged by food consumption, growth, mortality or other characteristic signs of intoxication (i.e., muscular signs or paralysis)	Hill and Carlisle, 1947
Guinea pigs/NS	6/group sex NS	saline	purified commercial	50 or 100 mg/day of 2,4-D sodium salt by intubation 10 daily doses in 12 days	139.8 or 252.6[c]	No treatment-related signs of intoxication or mortality	Hill and Carlisle, 1947
Dogs/mongrel 7-15 kg	Control, 2 f; 2 mg/kg, 1 M, 1 f; 5 mg/kg, 1 M, 1 f; 10 mg/kg, 3 M; 20 mg/kg, 3 M, 1 f	capsule	98.5% commercial	0, 2, 5, 10 or 20 mg/kg/day, 5 days/ week x 13 weeks	0, 1.4, 3.6, 7.1 or 14.3	1.4, 3.6 and 7.1 mg/kg caused no signs of toxicity, no significant effects on body weight, organ weight, gross and histological appearance of organs, and hematologic values; 14.3 mg/kg caused death of 3 by day 49, ataxia, stiffness of hind legs, difficulty in swallowing, no significant lesions	Drill and Hiratzka, 1953

[a]When the chemical was given in the diet, the dose was calculated by assuming that a young rat or dog consumes the equivalent of 0.1 or 0.029, respectively, of its body weight per day as food.

[b]Time-weighted average dose

[c]2,4-D acid-equivalent dose

NS = Not specified; NA = not applicable because food was not eaten

In a related study, groups of five young adult female rats (strain not specified) were maintained on diets that contained 0, 100, 300 or 1000 ppm 2,4-D in the diet for 113 days (Rowe and Hymas, 1954). If it is assumed that young rats consume 10% of their weight in food per day, the corresponding daily doses are 0, 10, 30 and 100 mg/kg/day. Rats that were exposed at the 1000 ppm level experienced excessive mortality (not quantified), depressed growth rate and slight cloudy swelling of the liver. These effects were not observed at the two lowest doses (see Table V-2). Groups of five rats that were given diets that contained higher concentrations of 2,4-D (3000 or 5000 ppm) were sacrificed after 12 days because they were not eating and were rapidly losing weight; examinations revealed increased liver and kidney weights and slight but unspecified pathologic changes.

Chang et al. (1974) reported that dietary administration of 2,4-D to rats at levels of 2000 ppm in the diet (~200 mg/kg/day) for 4-7 weeks produced a slight increase in liver glycogen content, a slight decrease in liver RNA content and slight decreases in absolute and relative liver weights, but no overt signs of toxicity (see Table V-2).

Administration of 0, 100, 200 or 400 ppm dietary 2,4-D (~0, 10, 20 or 40 mg/kg/day, respectively) to groups of seven rats for 1 month did not adversely affect food intake or rate of growth, or elicit characteristic signs of intoxication (skeletal muscular signs or paralysis) (Hill and Carlisle, 1947). Dietary administration of 2,4-D at a level of 100 ppm for 21 days and subsequently 1000 ppm for 10 days (average total dose ~39.0 mg/kg/day) was similarly nontoxic for rats. Groups of six guinea pigs that

were given 10 daily doses of 50 or 100 mg 2,4-D in 12 days (~88 or 177 mg/kg/day) by intubation also did not develop characteristic evidence of intoxication.

Drill and Hiratzka (1953) administered 2,4-D orally in capsules to groups of 2-4 dogs at doses of 0, 2, 5, 10 or 20 mg/kg/day, 5 days/week, for 13 weeks. When adjusted for a 7-day week, the respective daily doses were 0, 1.4, 3.6, 7.1 and 14.3 mg/kg/day. As detailed in Table V-2, toxic effects were only observed at the high dose. Treatment at 20 mg/kg/day produced death in 3/4 dogs between days 18 and 49, and symptoms in the moribund animals included hind leg stiffness, ataxia, weakness, gum bleeding and difficulty in chewing and swallowing. A terminal decrease in the percentage of blood lymphocytes was noted in the three dogs that died, but significant effects on the hemoglobin, red cell count or total white cell count were not observed. The dog that survived 2,4-D treatment at the high dose, as well as the dogs exposed to the lower levels of 2,4-D, showed no significant hematologic, gross or histopathologic effects.

In a study of limited design, pigs (Lantras strain, 18-25 kg, 8-12 weeks old) were administered 50 mg/kg commercial grade 2,4-D triethanolamine (water solvent) or 2,4-D butyl ester (diluted in petroleum solvent and emulsified in water) by intubation (Bjorklund and Erne, 1966). The compounds were administered at different frequencies and durations, but only one pig per exposure schedule was tested. Four pigs were exposed to the triethanolamine salt for 3 doses in 5 days or 15 doses in 20 days, and three pigs were exposed to the butyl ester for 5 doses in 8 days or 12 doses in 17 days.

Effects (e.g., anorexia, diarrhea, catarrhal gastroenteritis, fatty degeneration in the kidneys) were noted in 3/4 pigs exposed to 2,4-D triethanolamine and in 2/3 pigs exposed to 2,4-D butyl ester. Single pigs exposed to fifty-one 50 mg/kg doses of triethanolamine salt, to 100 mg/kg triethanolamine salt (3 doses in 7 days or 7 doses in 9 days), to twenty-three 50 mg/kg doses of butyl ester in 39 days, or to 300 mg/kg butyl ester (2 doses in 4 days or 3 doses in 6 days) exhibited similar effects.

Repeated s.c. injections of 93 mg/kg levels of 2,4-D sodium salt daily for 90 days did not produce significant symptoms in treated mice and histological examination did not show abnormalities (Bucher, 1946). Dilated lungs, liver and kidneys, however, were noted in moribund animals injected with 200 mg/kg levels of compound; the significance of these changes is unknown.

Effects of s.c. injected 2,4-D sodium salt on the thyroid gland of treated rats have been reported (Florsheim and Velcoff, 1962; Florsheim et al., 1963). These investigators showed that thyroid weight was decreased following seven daily injections of 2,4-D at a level of 100 mg/kg. Administration of 2,4-D at 80 mg/kg over this period increased radioactive iodine uptake by the thyroid, lowered the binding of radiolabeled thyroxine by serum proteins, and increased the amount of radiolabeled compound in the liver of treated rats.

Desi et al. (1962) described the toxic effects of 2,4-D or the nervous system of rats administered lethal doses of the compound i.p. Animals injected daily with 200 mg/kg of 2,4-D (form not specified) died within 6

days. Progressively decreased conditioned reflex responses were observed over this period, as well as the appearance of large slow waves in the EEG. Histological examination indicated that demyelinization was present in the dorsal portion of the spinal tract. Within 10-15 minutes following a single i.p. injection of the compound, EEG changes were observed (decreased cerebral and reticular desynchronization); recovery was seen in ~1 hour. The authors postulate that the neurological effects produced by 2,4-D in this study are due initially to action of the compound on the reticular formation, followed by later effects on cerebral tissue. Histological examination, however, failed to show any morphological changes in the cortex or subcortical regions of treated animals. The demyelinization observed in the spinal cord may be responsible for the hind limb paralysis noted by other investigators after poisoning of animals with 2,4-D.

The subchronic dermal toxicity of the dimethylamine salt and the isooctyl and butyl esters of 2,4-D has been studied in rabbits (Kay et al., 1965). Solutions containing the salt or esters at levels corresponding to 0.6 and 3.1% 2,4-D were applied to gauze patches, occluded, and left in place for 7-hour periods, 5 times/week for 3 weeks. No nervous system damage, body weight effects or hematological changes were observed following these levels of treatment. Local skin inflammation was noted in both control and treated animals, but subepithelial fibrosis and mononuclear infiltration appeared to be increased only in those animals treated with the oil dilutions of either of the 2,4-D esters.

Chronic Toxicity

Hansen et al. (1971) conducted 2-year feeding studies with technical grade (96.7% pure) 2,4-D in Osborne-Mendel rats. In the rat study, 25 animals of each sex were exposed to 0, 5, 25, 125, 625 or 1250 ppm 2,4-D in the diet (or 0, 0.25, 1.25, 6.25, 31.25 or 62.5 mg/kg bw//day, respectively, assuming a rat consu[illegible]% of its body weight per day) from 3 weeks of age. At the conclusion of treatment, all rats were autopsied, but comprehensive histopathologic examinations were performed only on 6 rats/sex from the high-dose and control groups; the liver, kidneys, spleen, ovaries or testes and other tissues that contained gross lesions were histologically examined in the remaining rats in the high exposure and control groups and in the rats at the other dose levels. Significant differences in survival, mean body weight and organ-to-body weight ratios (liver, kidney, heart, spleen or testes) were not found between any of the treated groups and the control group during the 2-year treatment or at the end of the study. Significant treatment-related pathologic effects were not observed and, as detailed in the Carcinogenicity Section, the incidence of tumors did not differ significantly between the groups. Several hematologic indices (hemoglobin, hematocrit, total white cell count) were similar in the treated and control groups, but the red blood cell count of the treated rats (1250, 625 and 5 ppm groups) showed a "tendency" toward macrocytosis, "very slight to slight" polychromasia, and "slight to moderate" hypochromasia. The tendency toward macrocytosis was reportedly not present and the other red cell abnormalities were of a "minor degree" in the control rats. The toxicological significance of these vaguely reported effects is unclear.

In a 2-generation reproduction study by Bjorklund and Erne (1966) that is also discussed in the Other Reproductive Effects Section, administration of 1000 ppm 2,4-D in the drinking water (~50-100 mg/kg/day) of rats (5/group) during pregnancy and for a further 10 months had no significant effects on the maternal animals (not specified) or offspring (clinical signs or malformations). Similar exposure of 22 weaned offspring (10 males, 12 females) for up to 2 years was also nontoxic as judged by normal clinical chemistry [indices were hematocrit, hemoglobin, plasma GOT, plasma elimination rate of 2,4-D (3 hours)], relative organ weights (heart, spleen, liver, kidneys, lungs, testes, ovaries), or gross or microscopic pathology. However, reduced food and water intake and consequent growth retardation, temporary diarrhea and poor general condition were observed. Other reproduction studies that are detailed in the Teratogenicity and Other Reproductive Effects Section reported that dietary exposure to 1500 ppm (~75 mg/kg bw) 2,4-D for 2 years (Hansen et al., 1971) and dietary exposure to 1000 ppm (~100 mg/kg) for 3 months (Gaines and Kimbrough, 1970) before mating and during pregnancy and lactation caused an increase in preweanling mortality.

Hansen et al. (1971) also fed 6- to 8-month-old beagle dogs (3 of each sex/group) 0, 10, 50, 100 or 500 ppm technical grade 2,4-D in the diet (~0, 0.29, 1.45, 2.9 or 14.5 mg/kg/day) for 2 years. Treatment-related effects based on observations of mortality as well as gross and microscopic tissue examinations were not indicated in any of the treated groups or the control group.

Santolucito (1975) reported EEG changes in a group of six squirrel monkeys (sex unspecified) that were exposed orally (method unspecified) to 0.2 mg 2,4-D/kg bw/day for 3 years. EEG recordings were apparently made only once, at the end of 3 years, at which time the treated monkeys were compared with seven concurrent controls. Changes during anaesthetized sleep included an increased proportion of high-frequency EEG waves and an increased number of zero potential crossovers per EEG recording. The data were obtained by on-line computer-assisted interval analysis of 5 minutes of each recording, but the toxicological significance of these changes is not known.

Carcinogenicity

Several studies have investigated the ability of 2,4-D and related compounds to produce tumors in laboratory rats and mice.

Bionetics Research Laboratories (Bionetics, 1968a; Innes et al., 1969) conducted a broad survey of the potential carcinogenic activity of several pesticides and industrial chemicals, including 2,4-D (90% pure) and its isopropyl (99% pure), butyl (99% pure) and isooctyl (97% pure) esters. Carcinogenic effects following chronic oral administration or a single s.c. injection were investigated in two strains of C57Bl/6 mice designated $B6C3F_1$ and $B6AKF_1$.

The oral administration regime consisted of intubation of the compound suspended in 0.5% gelatin to groups of 18 male and 18 female mice from 7-28 days of age, followed by dietary administration for ~10-24 months. Both strains of mice were given 46.4 mg/kg initial bw of the isopropyl, butyl or

isooctyl ester by intubation, followed by dietary concentrations of 111, 149 and 130 ppm (~14.4, 19.4 and 16.9 mg/kg/day), respectively, for 73-83 weeks. $B6AKF_1$ mice received 2,4-D by intubation at 46.4 mg/kg initial body weight, followed by 149 ppm (~19.4 mg/kg/day) in the feed for 75 weeks. Both strains received 2,4-D by intubation at 100 mg/kg initial body weight, followed by 323 ppm (~42 mg/kg/day) in the feed for 73-80 weeks. Groups of 36 male and 36 female mice of both species received 0.5% gelatin or no treatment at all. These control groups were assigned randomly to rooms housing treated animals.

Following the treatment period, all surviving mice were grossly examined on dissection, and the tissues from livers, spleens, kidneys, adrenals, stomachs, intestines and genitalia, which had been fixed and stained, were examined microscopically by a pathologist. In addition, mice that were killed when moribund were given a gross pathologic examination, and tissues were examined microscopically as deemed appropriate (criteria unspecified). No statistically significant ($p<0.05$) increase in tumor incidence over controls was found when any group or combination of groups was compared. Because of the relatively small number of animals/group and the limited nature of the histopathologic examinations, weak carcinogenic effects might not have been detected.

Groups of 18 male and 18 female mice of both strains received a single s.c. injection (neck) of 215 or 464 mg 2,4-D/kg bw dissolved in dimethylsulfoxide (DMSO) at age 28 days and were observed for 78 weeks. Similar groups of mice received a single s.c. injection of 100, 21.5 or 21.5 mg/kg bw of the isopropyl, isobutyl or isooctyl ester of 2,4-D (in corn oil),

group and the high dose level with respect to the number of male rats with malignant tumors (Table V-3). The tumors observed, however, were not associated with any specific tissue, but were randomly distributed and were of types usually observed in aging Osborne-Mendel rats.

In an unpublished evaluation of this study, Reuber (1979) reexamined the original histopathology sections, and reported substantially more tumors among dosed animals than had been reported by Hansen et al. (1971). A more detailed histopathological examination of all tissues and especially of those in the lower dose animals was apparently deemed necessary by this author. Reuber (1979) reported a greater number of lymphosarcomas in treated rats of both sexes and found a significant (Fisher exact $p<0.05$) increase in the incidence of this tumor among female rats at all five dose levels. The differences in tumor incidence reported by Hansen et al. (1971) and Reuber (1979) might be resolved if an independent reexamination of the tissue sections were performed.

The amine salt of 2,4-D has been tested for carcinogenic activity in rats and mice following oral administration (Archipov and Kozlova, 1974). Rats, 120 males and 45 females, were fed 2,4-D amine at one-tenth the LD_{50} level (not specified) for life. A similar dietary level of compound was fed to a group of 100 mice for their lifespan. Neither species of test animal developed a significant increase in tumors following oral treatment. The only tumors identified were a mammary fibroadenoma and a hemangioma of the mesenterium in two treated rats and a mammary fibroadenoma in one untreated rat. These investigators also reported that long-term application of 2 drops/week of a 10% acetone solution of compound to the shaved backs of 100 mice failed to produce tumors. When this treatment with 2,4-D was preceded

TABLE V-3

Tumor Incidence in Rats Fed 2,4-D[a]

Dose (ppm)	Rats[b] with Tumors		Rats[b] with Malignant Tumors	
	Males	Females	Males	Females
0	3	12	1	5
5	5	9	2	6
25	5	13	4	3
125	6	14	2	5
625	6	17	5	3
1250	7	15	6[c]	8

[a]Source: Hansen et al., 1971

[b]Rats/sex/dose = 25

[c]$p<0.05$

by dermal application of 1 drop of a 5% solution of 3-methylcholanthrene (MCA), an increase (0-17.7%) in skin papillomas was observed. The authors concluded that 2,4-D showed significant cocarcinogenic activity; this protocol suggests that 2,4-D was tested for tumor promoter activity. No treatment of MCA-initiated control animals was carried out over the 20-month test period. It is unclear how long after 2,4-D treatment began these papillomas developed.

An additional animal bioassay (Industry Task Force, 1985) in rats and mice has been provided to EPA, although the bioassay is not independently evaluated by the ORD Carcinogen Assessment Group in this assessment, the study will be reviewed prior to finalization of this document. On an interim basis therefore this document reports the assessment position. According to EPA, 1988, (EPA Press Release, Tuesday March 15, 1988; 2,4-D) "A rat bioassay (Industry Task Force, 1985) found an apparent treatment-related increased incidence of brain tumors in males at the highest dose level. However, the increased incidence of tumors seen in the male rats at the highest dose level was not statistically significant when compared to control male rats, although a marginally statistically significant trend was observed. No tumor response was seen in female rats or mice". The Office of Pesticides tentatively concluded that the tumor induction from the rat study provided limited evidence of carcinogenicity in animals. In June 1987, the FIFRA Scientific Advisory Panel reviewed the Office of Pesticides classification (limited evidence) and advised that the evidence should be viewed as equivocal and recommended additional testing. The Office of Pesticides has accepted the assessment of the animal data by the SAB.

Available data from laboratory animals have not provided a sufficient demonstration of carcinogenicity of 2,4-D, although increased tumor production is suggested. This question cannot be adequately resolved until more compelling evidence is available.

Epidemiology studies have associated excess tumor incidence in humans with mixed exposures to chlorophenoxy herbicides, including 2,4,5-T (which may be contaminated with 2,3,7,8-TCDD) and 2,4-D (which is not contaminated with this dioxin isomer). These studies do not specifically attribute carcinogenic effects to 2,4-D alone, and are summarized in the Subchronic and Chronic Effects Section in Chapter VI.

Mutagenicity

The mutagenic activity of 2,4-D has been investigated in a number of organisms including bacteria, yeast, Drosophila melanogaster, algae and several species of plants (Tables V-4 and V-5). Mammalian studies relating to the mutagenicity of 2,4-D have included the micronucleus assay, the dominant lethal assay, inhibition of testicular DNA synthesis and several in vitro assays of peripheral blood lymphocytes or cell lines treated with 2,4-D.

Investigations of the mutagenicity using microorganisms have generally failed to show activity of the compound. These negative results include testing with Saccharomyces cerevisiae (Fahrig, 1974), Salmonella typhimurium (Andersen et al., 1972; Styles, 1973; Andersen and Styles, 1978; Ercegovich and Rashid, 1977; Commoner, 1976; Zetterberg et al., 1977), T_4 bacteriophage (Anderson et al., 1972), Bacillus subtilis (Shirasu et al., 1976) and Escherichia coli (Fiscor and Piccolo, 1972; Ercegovich and Rashid, 1977).

TABLE V-4

Mutagenicity Testing of 2,4-D

Assay	Strain	Compound	Concentration	Mammalian Activation	Application	Response	Reference
Gene conversion Saccharomyces cerevisiae	D_4	2,4-D	NR	no	liquid holding, neutral pH	-	Fahrig, 1974
	D_4	2,4-D	2 mL of U-46 solution	no	liquid, low pH	+	Siebert and Lemperle, 1974
	D_4	2,4-D	200 mg/kg, oral intubation	host-mediated, CBA mice	1.p. injection of bacteria	-	Zetterberg et al., 1977
	D_4	2,4-D	0.6 mg/L	no	liquid, low pH	+	Zetterberg et al., 1977
Gene combination Saccharomyces cerevisiae	D_5	2,4-D	0.3 mg/mL	no	liquid, low pH	+	Zetterberg et al., 1977
	D_5	2,4-D	0.2 mg/L	no	liquid, low pH	-	Zetterberg et al., 1977
	RAD 18	2,4-D	0.2 mg/L	no	liquid, low pH	+	Zetterberg, 1978
Reversion, Salmonella typhimurium	TA1535	2,4-D	0.3-0.8 mg/mL	no	liquid	-	Zetterberg et al., 1977
	TA1538	2,4-D	0.3-0.8 mg/mL	no	liquid	-	Zetterberg et al., 1977
	TA1530	2,4-D	200 mg/kg, oral intubation	host-mediated, CBA mice	1.p. injection of bacteria	-	Zetterberg et al., 1977
	TA1531	2,4-D	200 mg/kg, oral intubation	host-mediated, CBA mice	1.p. injection of bacteria	-	Zetterberg et al., 1977
	his mutants	2,4-D	1-5 µL technical solution	no	agar overlay	-	Anderson et al., 1972
	TA98	2,4-D acetate	4-2500 µg/plate	Aroclor induced rat S-9	agar	-	Anderson and Styles, 1978
	TA100	2,4-D acetate	4-2500 µg/plate	Aroclor induced rat S-9	agar	-	Anderson and Styles, 1978
	TA1535	2,4-D acetate	4-2500 µg/plate	Aroclor induced rat S-9	agar	-	Anderson and Styles, 1978

TABLE V-4 (cont.)

Assay	Strain	Compound	Concentration	Mammalian Activation	Application	Response	Reference
Reversion, Salmonella typhimurium	TA1538	2,4-D acetate	4-2500 µg/plate	Aroclor induced rat S-9	agar	-	Anderson and Styles, 1978
	his mutants	2,4-D	NR, oral	modified host-mediated, rat	bacteria incubated in serum from treated rats and plated	-	Styles, 1973
	his mutants	2,4-D	NR	NR	NR	-	Ercegovich and Rashid, 1977
	his mutants	2,4-D	10 µg/plate		agar overlay	-	Commoner, 1976
Reversion, bacteriophage	T_4, rII mutants	2,4-D technical grade	50 µg/plate	no	tryptone plates	-	Anderson et al., 1972
Point mutation, bacteriophage	T_4	2,4-D technical grade	50 µg/plate	no	agar	-	Anderson et al., 1972
DNA modification, Escherchia coli	DNA polymerase deficient	2,4-D	NR	NR	disc	-	Rosenkranz and Leifer, 1979
	E 3110 and p 3478	2,4-D	5 mg/plate	no	disc	+	Simmon, 1979
	WP2 try	2,4-D	20-25 µg/plate	NR	disc	-	Nagy and Antoni, 1975
	5 strains	2,4-D amine, dicamba, dimethylamine	NR	NR	disc	-	Fiscor and Piccolo, 1972
DNA cell binding, Escherchia coli	Q13	2,4-D	20-200 µM	± rat S-9 or egg-white lysozomes	liquid	+	Kubinski et al., 1981
DNA modification, Bacillus subtilis	H17 and M45	2,4-D	5 mg/plate	no	disc	+	Simmon, 1979
Recombination, Bacillus subtilis	H17 Rec+	2,4-D	0.02 mg/plate	no	disc	-	Shirasu et al., 1976
	M45 Rec-	2,4-D	0.02 mg/plate	no	disc	-	Shirasu et al., 1976
Recessive lethals, Drosophila melanogaster	Berlin K males, In(1)$SC^{S1}lSC^{8R}$, S,$SC^{S1}SC^{8}w^{a}8$ females	2,4-D	9 mM	no	oral, 3 days	-	Vogel and Chandler, 1974

TABLE V-4 (cont.)

Assay	Strain	Compound	Concentration	Mammalian Activation	Application	Response	Reference
Recessive lethals, Drosophila melanogaster		2,4-D diethylamine	0.08-8 mg/mL	NR	NR	-	Berin et al., 1973
	ywsn/ywsn x ywsn/y+Y	2,4-D	100 ppm	no	larval feeding	+	Magnusson et al., 1977
	stable white locus	2,4-D	100 ppm	no	larval feeding	+	Rasmusson and Svahlin, 1978
	stable white locus	2,4-D	50 ppm	no	larval feeding	-	Rasmusson and Svahlin, 1978
	unstable white locus	2,4-D	100 ppm	no	larval feeding	+	Rasmusson and Svahlin, 1978
	unstable white locus	2,4-D	50 ppm	no	larval feeding	-	Rasmusson and Svahlin, 1978
Somatic mutations, Drosophila melanogaster	stable white locus	2,4-D	25 ppm	no	larval feeding	-	Rasmusson and Svahlin, 1978
	unstable white locus	2,4-D	25 ppm	no	larval feeding	+	Rasmusson and Svahlin, 1978
Nondisjunction, Drosophila melanogaster	$yw^2f/yw^2fxyw^2f/y^+YB^S$	2,4-D	100 ppm	no	larval feeding	-	Ramel and Magnusson, 1979
Sex chromosome loss, Drosophila melanogaster	$yw^2f/yw^2fxyw^2f/y^+YB^S$	2,4-D	100 ppm	no	larval feeding	-	Ramel and Magnusson, 1979
Ouabain resistant mutation, hamster lung cell cultures	V-79 cell line	2,4-D fluid	10 µm	none added	liquid	+	Ahmed et al., 1977
Unscheduled DNA synthesis, transformed human fibroblasts SV-40	VA-4 cell line	2,4-D fluid	1-1000 µm	none added	liquid	+	Ahmed et al., 1977
	VA-4 cell line	2,4-D fluid	1-1000 µm	rat S-9	liquid	+	Ahmed et al., 1977
Cell transformation human lung cell culture	WI-38	2,4-D acetate	0.08-250 µg/mL	none added	liquid	-	Styles, 1977
	WI-38	2,4-D acetate	0.08-250 µg/mL	rat S-9	liquid	-	Styles, 1977

TABLE V-4 (cont.)

Assay	Strain	Compound	Concentration	Mammalian Activation	Application	Response	Reference
Cell transformation Syrian hamster kidney	BKH-21	2,4-D acetate	0.08-250 µg/ml	none added	liquid	-	Styles, 1977
	BHK-21	2,4-D acetate	0.08-250 µg/ml	rat S-9	liquid	-	Styles, 1977
Micronucleus assay, mouse	male CBA	2,4-D	100 mg/kg	in vivo	i.p. injection	-	Jenssen and Renberg, 1976
Dominant lethal assay, mouse	ICR/Ha Swiss	2,4-D	125 mg/kg	in vivo	i.p. injection	-	Epstein et al., 1972
Chromosomal aberrations, mouse bone marrow	linear white	2,4-D	300 mg/kg	in vivo	i.p. injection	+	Pilinskaya et al., 1976
	linear white	2,4-D	50 mg/kg	in vivo	i.p. injection	-	Pilinskaya et al., 1976
Chromosomal aberrations, human blood lymphocytes	NR	2,4-D	20 µg/ml	none added	medium	+	Pilinskaya et al., 1976
	NR	2,4-D	2 µg/ml	none added	medium	-	Pilinskaya et al., 1976
Sister chromatid exchange, human blood lymphocytes	NR	2,4-D	10-60 µg/ml	none added	medium	+	Korte and Jalal, 1982
	NR	2,4-D	0.2 µg/ml	none added	medium	-	Korte and Jalal, 1982
Unscheduled DNA synthesis, primary rat hepatocyte cultures	F344	2,4-D	0.5-1000 nm/ml	present in hepatocyte culture	medium	-	Probst et al., 1981
Chromosomal aberrations, human blood lymphocytes	NR	2,4-D	50-60 µg/ml	none	medium	+	Korte and Jalal, 1982
	NR	2,4-D	0.2-40 µg/ml	none	medium	-	Korte and Jalal, 1982
Chromosomal aberrations, embryonic bovine kidney cells	NR	2,4-D	1-1000 µg/ml	none	medium	-	Bongso and Basrur, 1973
Inhibition of thymidine incorporation into testicular DNA, mice	NR	2,4-D	200 mg/kg	in vivo	oral	+	Seiler, 1979

NR = Not reported

TABLE V-5

Mutagenicity Testing of 2,4-D in Plants

Test	Compound	Concentration	Application	Mutagenicity	Assay	Reference
Barley	2,4-D	1.5 mL	injection into spike	+	phenotypic mutants	Demerec, 1954
Barley	2,4-D	100 ppm	9-hour treatment of seeds	+	chlorophyll mutation	Khalatkar and Bhargava, 1982
Barley	2,4-D mixed butyl ester	200 ppm	6-hour treatment of seeds	+	chlorophyll mutation	Mohandas and Grant, 1972
Wheat	2,4-D amine	8 ounces	pre-boot and tillering stages	+	phenotypic mutants	Sandhu, 1957
Wheat, barley	2,4-D ester	12 ounces acid/ acre	spray	+	chromosome aberrations	Unrau and Larter, 1951
5 species, weed	2,4-D amine	907 g acid/0.4 ha	spray	+	chromosome aberrations	Tomkins and Grant, 1976
Tobacco	2,4-D	0.4 ppm, 120 hours	medium	+	chromosome aberrations	Nuti-Ronchi et al., 1976
Bean	2,4-D	0.39 ppm	spray	+	chromosome aberrations	Amer and Ali, 1974
Pea	2,4-D	40 ppm, 8-12 hours	seedling	-	chromosome aberrations	Muhling et al., 1960
Geranium	2,4-D	10^{-8} M	liquid	+	somatic mutation	Pohlheim et al., 1977
Onion	2,4-D mixed butyl esters	50 ppm, 6 hours	root application	+	chromosome aberrations	Mohandas and Grant, 1972
Carrot	2,4-D	0.1 mg/L	medium	+	chromosome aberrations	Bayliss, 1973
Helianthus annuus	2,4-D	>10 ppm	NR	+	chromosome aberrations	Siddiqui et al., 1982

NR = Not reported

Positive mutagenic effects of 2,4-D were reported by Siebert and Lemperle (1974) in S. cerevisiae following treatment of cell suspensions with 2 mℓ of commercial U-46 D-Fluid (2,4-D acid) at pH 4.5; this concentration of 2,4-D produced toxicity in treated cells. Mutagenic effects have also been reported by Simmon (1979) for DNA repair-deficient strains of E. coli and B. subtilis treated with 2,4-D (5 mg/plate). Clarification of these varied results has been provided by the work of Zetterberg et al. (1977), who illustrated a definite pH-dependency in obtaining mutagenic effects of 2,4-D in S. cerevisiae. At pH 4.5, cells showed a dose-dependent increase in gene conversion and cellular toxicity at 2,4-D concentrations from 0.1-0.6 mg/mℓ, while at neutral pH, neither effect was observed. The authors indicate that, at neutral pH, 2,4-D is primarily in a dissociated (ionized) state and does not readily penetrate cell membranes. Zetterberg (1978) also showed a pH-dependent increase in S. cerevisiae revertants exposed to 0.2 mg/mℓ of 2,4-D. Kubinski et al. (1981) reported that binding of 2,4-D to E. coli Q13 DNA was enhanced at close to neutral pH (7.2-7.4) in the presence of a cell membrane disruptor (egg-white lysozymes) or a metabolic activator (rat liver enzymes).

Detection of sex-linked recessive lethals in Drosophila melanogaster treated with 2,4-D has been used as a mutagenicity assay by several investigators (Vogel and Chandler, 1974; Magnusson et al., 1977; Rasmusson and Svahlin, 1978). Vogel and Chandler (1974) were unable to show a statistically significant increase in recessive lethals after larval feeding of 9 mM 2,4-D for 3 days; examination was for the F_1 generation. Positive mutagenic effects were reported by Magnusson et al. (1977) following 15-day larval feeding with 100 ppm 2,4-D. Lethals observed in F_1 were not significantly increased, but pooled data from the F_1 and F_2 generations

showed a 2- to 3-fold increase in lethals over controls. These investigators were unable to show chemically induced nondisjunction or loss of sex chromosomes in D. melanogaster treated with the same level of 2,4-D. This assay, however, is less sensitive than the production of recessive lethals for screening of mutagenic agents. Rasmusson and Svahlin (1978) compared phenotypic changes in eye color induced by 2,4-D in two strains of D. melanogaster. They observed that larval exposure to 25 ppm of 2,4-D produced a significant increase in mutations for an unstable D. melanogaster strain (foreign DNA inserted in the structural gene), but failed to do so for a stable strain. The positive control, ethyl methane sulfonate, at 500 ppm levels produced mutations in both strains.

Studies involving 2,4-D and its salts and esters applied to various plant species have indicated chromosomal effects of the compounds. Grant (1978) has argued that the effects of pesticides in plants correlate well with effects in cultured mammalian cells and, therefore, such effects in plants should be considered to indicate possible mutagenic activity of 2,4-D in mammals. Positive mutagenic effects in plants treated with 2,4-D have been reported by many investigators; these have included experiments with barley (Denward, 1954; Mohandas and Grant, 1972; Khalatkar and Bhargava, 1982), wheat (Sandhu, 1957), wheat and barley (Unrau and Larter, 1951), tobacco (Nuti-Ronchi et al., 1976), beans (Amer and Ali, 1974), carrots (Bayliss, 1973), geranium (Pohlheim et al., 1977) and several weed species (Tomkins and Grant, 1976). Denward (1954) recorded seven mutants in the second generation progeny of barley plants that had been injected near the spikes with 1.5 mℓ of a 2,4-D solution (concentration unknown). Spraying of wheat and barley plants at various stages of growth with 2,4-D ester at a level equivalent to 12 ounces of 2,4-D acid/acre has been reported by Unrau

and Larter (1951) to produce a number of meiotic abnormalities. Chromosome aberrations included bridges, fragments, aneuploidy and polyploidy, chain and ring formation, and sticky chromosomes. The incidence of these chromosome effects at different times after emergence indicated that sensitivity to 2,4-D changes throughout the growth cycle. Sandhu (1957) reported a higher number of off-plants in progeny of barley treated with 8 ounces of 2,4-D amine solution. Khalatkar and Bhargava (1982) reported the induction of mitotic and meiotic chromosomal aberrations, pollen sterility, spike and seed morphological alterations in the M_1 generation and chlorophyll-deficient mutations in the M_2 generation grown from barley seeds soaked in a 100 ppm solution of 2,4-D for 9 hours.

Treatment of germinated barley seeds with 200 ppm of a commercial herbicide preparation of the mixed butyl esters of 2,4-D for 6 hours has been reported to produce five chlorophyll mutants in M_2 seedlings during field trials (Mohandas and Grant, 1972). These investigators also noted chromosome effects similar to those described by Denward (1954) in root tip cells of Allium cepa (onion) treated with 50-100 ppm of 2,4-D for 6 hours. Data on the scoring of these chromosome effects were not presented. Two other species of plants also showed root tip effects after application of this commercial 2,4-D ester preparation, while three species tested failed to show an increase in abnormal cells. The types of aberrations described, including the finding of C-mitosis, suggest that 2,4-D may interact with the spindle apparatus during cell division. Seiler (1978) points out that cereals are generally insensitive to the auxin-like activity of chlorophenoxy acetic acids, but whether the in vitro effects observed in plant cells treated with 2,4-D are the result of physiological effects on cell growth or direct effects on chromosomal material is not clear.

Tomkins and Grant (1976) found increased chromosome aberrations in 5 of 12 weed species sprayed with a 2,4-D amine solution at a dose of 907 g of 2,4-D acid equivalent per 0.4 ha. These included chromosome fragments, bridges and lagging chromosomes. Similar chromosome effects were observed by Amer and Ali (1974) in bean plants following spraying with 0.39 ppm 2,4-D for 5 days. These workers found that the growth stage of the plants influenced the sensitivity to 2,4-D treatment. Disturbed metaphases and anaphases indicated effects on the mitotic spindle. Siddiqui et al. (1982) observed abnormalities in meiotic chromosomes of Helianthus annuus treated with 10 or 20 ppm of 2,4-D, but not 5 ppm. These aberrations included chromosome pairing failure, stickiness, bridges, irregular separation, laggards and fragments. Muhling et al. (1960) were unable to show increases in chromosome breaks following treatment of pea seedling roots with 40 ppm levels of a 2,4-D solution; however, colchicine-like effects observed in mitotic preparations suggested that 2,4-D may have acted as a spindle poison. Bayliss (1973) noted similar chromosomal and mitotic abnormalities in root tip preparations of carrots treated in vitro with 0.1 mg/ℓ of 2,4-D. Increased chromosome breakage in tissue cultures of tobacco plant cells following in vitro exposure to 0.4 ppm 2,4-D has also been reported by Nuti-Ronchi et al. (1976). These effects were also produced in this habituated line of plant cells by the addition of a synthetic auxin, kinetin. Nonhabituated cells that required the presence of growth factor for survival in culture showed no chromosome breakage from treatment with 2,4-D or kinetin.

Assays using in vivo mammalian metabolic activation have failed to show mutagenic activity of 2,4-D, including the host mediated assay with S. typhimurium (Styles, 1973) and the host mediated assay with S. cerevisiae

(Zetterberg et al., 1977). Similarly, two mammalian assays, the dominant lethal assay (Epstein et al., 1972) and the micronucleus test (Jenssen and Renberg, 1976), have not demonstrated genotoxic effects. This lack of activity in mammalian systems may correlate with the finding of Jenssen and Renberg (1976) that <5% of a 100 mg/kg dose of 2,4-D injected i.p. into mice was available for penetration into bone marrow cells within 24 hours. Pilinskaya et al. (1976), however, reported an increase in chromosome aberrations of bone marrow cells from mice treated with 100 or 300 mg/kg oral doses of 2,4-D. No data on the scoring of these cells were presented, but the authors stated that the chromosome aberrations were primarily single fragments. The purity of the 2,4-D and the vehicle used in this study were not described.

Conflicting results for the genotoxicity of 2,4-D have been observed in several in vitro assays using mammalian cells. Probst et al. (1981) reported that 2,4-D did not stimulate unscheduled DNA synthesis in primary rat hepatocyte cultures that retain metabolic capability. Murakami and Fukami (1980, 1982) reported the absence of 2,4-D binding to human embryonic DNA in cultured cells. Ahmed et al. (1977), however, found an increase in oubain resistant mutants following treatment of cultured V-79 Chinese hamster lung cells with a 2,4-D concentration of 10 μM. Further, they found increased unscheduled DNA synthesis and increased bromodeoxyuridine photolysis in SV-40 transformed human fibroblasts treated with or without a source of metabolic activation using the same concentration range of 2,4-D. Calculations to determine the number of breaks produced in 2,4-D-treated cells in the photolysis assay indicated very little increase as the dose of 2,4-D was increased from 10-100 μM. Styles (1977) was unable to show increased transformation of cultured baby hamster kidney or human lung cell lines

treated *in vitro* with or without a source of metabolic activation at concentrations of 0.08-250 µg 2,4-D/mℓ.

Peripheral blood lymphocytes have been cultured in the presence of 2,4-D and scored for chromosome damage (Bongso and Basrur, 1973; Pilinskaya et al., 1976; Korte and Jalal, 1982). Pilinskaya et al. (1976) reported an increase in chromosome aberrations following treatment of cultured human lymphocytes with 20 µg/mℓ. Data on controls were not presented, nor were scoring data reported. Bovine peripheral blood cells exposed to 10-1000 ppm levels of 2,4-D showed altered mitosis and an elevated mitotic index, but no chromosomal aberrations (Bongso and Basrur, 1973).

Korte and Jalal (1982) incubated cultured human blood cells with 0.2-60 µg 2,4-D/mℓ for 48 hours and examined the chromosomes for evidence of aberration and sister chromatid exchange. They observed statistically significant increases in gaps and deletions in lymphocytes treated with 50 and 60 µg/mℓ ($p=0.05$), and in sister chromatid exchanges in lymphocytes treated with 10, 20, 30, 40, 50 and 60 µg/mℓ ($p=0.05$) over untreated controls.

A recent report has found that oral administration of 2,4-D to mice inhibited thymidine incorporation into testicular DNA (Seiler, 1979). This inhibition was observed when 200 mg/kg of 2,4-D was administered to mice 1 hour after an i.p. injection of ^{14}C-thymidine. The author noted that the order of mutagenic activity (MCPA > 2,4,5-T > 2,4-D) suggested by the work of other investigators was also observed in this assay (which has not been validated as a mutagenicity screening test).

These studies suggest that 2,4-D may have mutagenic activity in certain systems; however, the general lack of positive genotoxic effects in vivo for mammalian assays may indicate that sufficient levels of 2,4-D are not able to reach the target tissues. No information is available on mammalian mutagenicity testing conducted with the esters of 2,4-D; these forms could theoretically show higher levels of penetration into target cells.

Teratogenicity and Other Reproductive Effects

Teratogenicity. The teratogenic and embryotoxic effects of 2,4-D and several derivatives of 2,4-D have been investigated in several species of laboratory animals. Overall, 2,4-D and its derivatives appear to be embryotoxic but only weakly teratogenic or nonteratogenic. The teratogenic or embryotoxic (or both) effects observed following oral administration of 2,4-D and its derivatives during gestation are summarized in Table V-6.

Courtney (1977) investigated the ability of 2,4-D (no dioxins detected) and several derivatives of 2,4-D (no dioxins detected), including isopropyl, n-butyl, isooctyl and propylene glycol butyl ether (PGBE) esters of 2,4-D, and 2,4-D butyric acid, to induce cleft palates in CD-1 mice. Daily gastric intubation of 2,4-D and the propylene glycol butyl ether (PGBE) and n-butyl ester derivatives were given at levels of 124 mg/kg/day of 2,4-D on days 7-15 of gestation and 221 mg/kg/day of 2,4-D on days 12-15 of gestation. The isooctyl and isopropyl esters and butyric acid derivatives were administered at 124 mg/kg/day of 2,4-D on days 7-15 of gestation for the two esters and on days 11-13 of gestation for butyric acid. The day of detection of a vaginal plug was taken as day 1 of gestation; the animals were killed on day 18 of gestation. Control animals were given daily gastric intubations of the vehicle, consisting of 0.1 mL corn oil:acetone (in a ratio of 9:1).

TABLE V-6

Teratogenicity of Orally Administered 2,4-D and Derivatives of 2,4-D

Compound	Species/ Strain	No. Dams at Start	Vehicle	Purity	Dosage/Exposure	Dose as mg/kg/day of 2,4-D	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
2,4-D	mice/CD-1	vehicle controls: 4 groups, 7-16/ group; treated: 8/low dose, 14/high dose	corn oil: acetone	90%	0.56 mM/kg on days 7 through 15 of gestation	124	2% cleft palate/litter as compared with 0% for controls; no other effect on fetal parameters; no effect on maternal weight gain; increase ($p<0.05$) in maternal relative liver weight	Courtney, 1977
					1.00 mM/kg on days 12 through 15 of gestation	221	6% cleft palate/litter as compared with 0% for controls; decreased ($p<0.05$) fetal weight among litters; no effect on maternal weight gain; increase ($p<0.05$) in maternal relative liver weight	
PGBE ester of 2,4-D	mice/CD-1	vehicle controls: 4 groups, 7-16/ group; treated: 10/low dose, 7/high dose	corn oil: acetone	99.9%	0.56 mM/kg on days 7 through 15 of gestation	124	8% cleft palate/litter as compared with 0% for controls; decreased ($p<0.05$) fetal weight among litters; no effect on maternal weight gain; increase ($p<0.05$) in maternal relative liver weight	Courtney, 1977
					1.00 mM/kg on days 12 through 15 of gestation	221	16% cleft palate/litter as compared with 0% for controls; decreased ($p<0.05$) fetal weight among litters; no effect on maternal weight gain or relative liver weight	
n-Butyl ester of 2,4-D	mice/CD-1	vehicle controls: 4 groups, 7-16/ group; treated: 9/dose level	corn oil: acetone	98.4%	0.56 mM/kg on days 7 through 15 of gestation	124	No effect on fetal parameters or on maternal weight gain or relative liver weight	Courtney, 1977
					1.00 mM/kg on days 12 through 15 of gestation	221	15% cleft palate/litter as compared with 0% for controls; decreased ($p<0.05$) fetal weight among litters; no effect on maternal weight gain; increase ($p<0.05$) in maternal relative liver weight	

TABLE V-6 (cont.)

Compound	Species/ Strain	No. Dams at Start	Vehicle	Purity	Dosage/Exposure	Dose as mg/kg/day of 2,4-D	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
Isopropyl ester of 2,4-D	mice/CD-1	vehicle controls: 4 groups, 7-16/ group; treated: 10	corn oil: acetone	99%	0.56 mM/kg on days 7 through 15 of gestation	124	No incidence of cleft palate; decreased ($p<0.05$) fetal weight among litters; no effect on maternal weight gain or relative liver weight	Courtney, 1977
Isooctyl ester of 2,4-D	mice/CD-1	vehicle controls: 4 groups, 7-16/ group; treated: 11	corn oil: acetone	96.8%	0.56 mM/kg on days 7 through 15 of gestation	124	No incidence of cleft palate; decreased ($p<0.05$) fetal weight among litters; no effect on maternal weight gain or relative liver weight	Courtney, 1977
Butyric acid of 2,4-D	mice/CD-1	vehicle controls: 4 groups, 7-16/ group; treated: 8	corn oil: acetone	99.6%	0.56 mM/kg on days 11 through 13 of gestation	124	No incidence of cleft palate; no effect on other fetal parameters; no effect on maternal weight gain; increase ($p<0.05$) in maternal relative liver weight	Courtney, 1977
2,4-D	rats/ Wistar	6-14/dose/expt.	corn oil or aqueous gelatin	no dioxins detected	0, 25, 50, 100, 150 mg/kg/day on days 6 through 15 of gestation	0, 25, 50, 100, 150	Dose-related increased fetal mortality and decreased fetal weight significant ($p<0.05$) at 100 and 150 mg/kg/day; increased incidence of skeletal malformations* among litters significant ($p<0.05$) at 25, 100 and 150 mg/kg/day as compared with controls; no effect on maternal body weights.	Khera and McKinley, 1972
Isooctyl ester of 2,4-D	rats/ Wistar	17 controls, 5-6 treated/dose	corn oil	NR	50 mg/kg/day on days 6 through 15 of gestation	33.2	NOAEL as judged by fetal mortality, fetal weights, occurrence of skeletal or visceral anomalies*	Khera and McKinley, 1972
					150 mg/kg/day on days 6 through 15 of gestation	99.5	Decreased ($p<0.05$) fetal weight among litters; increased ($p<0.05$) incidence of skeletal malformations* among litters; no effect on maternal body weights	

Compound	Species/ Strain	No. Dams at Start	Vehicle	Purity	Dosage/Exposure	Dose as mg/kg/day of 2,4-D	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
Butyl ester of 2,4-D	rats/ Wistar	17 controls, 4-5 treated/dose	corn oil	NR	50 mg/kg/day on days 6 through 15 of gestation	40	NOAEL as judged by fetal mortality, fetal weights, occurrence of skeletal or visceral anomalies*	Khera and McKinley, 1972
					150 mg/kg/day on days 6 through 15 of gestation	120	Increased ($p<0.05$) fetal mortality among litters; decreased ($p<0.05$) fetal weight among litters; increased ($p<0.05$) incidence of skeletal malformations* among litters; no effect on maternal body weights	
Butoxy-ethanol ester of 2,4-D	rats/ Wistar	15 controls, 8-9 treated/dose	corn oil	NR	50 mg/kg/day on days 6 through 15 of gestation	34.4	NOAEL as judged by fetal mortality, fetal weights, occurrence of skeletal or visceral anomalies*	Khera and McKinley, 1972
					150 mg/kg/day on days 6 through 15 of gestation	103.2	Increased ($p<0.05$) incidence of skeletal malformations* among litters; no effect on maternal body weights	
Dimethyl-amine salt of 2,4-D	rats/ Wistar	15 controls, 7-10 treated/dose	corn oil	NR	100 mg/kg/day on days 6 through 15 of gestation	41.8	NOAEL as judged by fetal mortality, fetal weights, occurrence of skeletal or visceral anomalies*	Khera and McKinley, 1972
					300 mg/kg/day on days 6 through 15 of gestation	125.5	Increased ($p<0.05$) incidence of skeletal malformations* among litters; effect on maternal body weights	
2,4-D	rats/ Sprague-Dawley	controls: 2 groups, 36 and 41; treated: 13-21/dose	corn oil	commercial grade	12.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	12.5	Increased ($p<0.05$) incidence of delayed ossification of skull bones among fetuses and litters; no effect on maternal body weight	Schwetz et al., 1971
					25 mg/kg/day 2,4-D on days 6 through 15 of gestation	25	No significant effect on fetuses or on maternal body weight	

TABLE V-6 (cont.)

Compound	Species/ Strain	No. Dams at Start	Vehicle	Purity	Dosage/Exposure	Dose as mg/kg/day of 2,4-D	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
2,4-D					50 mg/kg/day 2,4-D on days 6 through 15 of gestation	50	Intermediate response between 2 lower dosage levels (12.5 and 25 mg/kg/day) and 2 higher dosage levels (75 and 87.5 mg/kg/day)	Schwetz et al., 1971
					75 or 87.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	75 or 87.5	Decreased ($p<0.05$) fetal weight among litters; increased ($p<0.05$) incidences of skeletal defects (including delayed ossification of sternebrae and/or skull, wavy ribs, lumbar ribs, missing sternebrae) among fetuses and litters; increased ($p<0.05$) incidence of subcutaneous edema among fetuses and litters; no effect on maternal body weight	
PGBE ester of 2,4-D	rats/ Sprague-Dawley	controls: 2 groups, 36 and 41; treated: 13-21/dose	corn oil	commercial grade	dose equimolar to 12.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	12.5	Increased ($p<0.05$) incidence of missing sternebrae among fetuses; no effect on maternal body weight	Schwetz et al., 1971
					dose equimolar to 25 mg/kg/day 2,4-D on days 6 through 15 of gestation	25	Increased ($p<0.05$) incidence of delayed ossification of skull bones among fetuses and litters; no effect on maternal body weight	
					dose equimolar to 50 mg/kg/day 2,4-D on days 6 through 15 of gestation	50	Intermediate response between 2 lower dosage levels (12.5 and 25 mg/kg/day) and 2 higher dosage levels (75 and 87.5 mg/kg/day)	

Compound	Species/ Strain	No. Dams at Start	Vehicle	Purity	Dosage/Exposure	Dose as mg/kg/day of 2,4-D	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
PGBE ester of 2,4-D					doses equimolar to 75 or 87.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	75 or 87.5	Decreased ($p<0.05$) fetal weight among litters; increased ($p<0.05$) incidences of skeletal defects (including delayed ossification of sternebrae and/or skull, wavy ribs, lumbar ribs, missing sternebrae) among fetuses and litters; increased ($p<0.05$) incidence of subcutaneous edema among fetuses and litters; no effect on maternal body weight	Schwetz et al., 1971
Isooctyl ester of 2,4-D	rats/ Sprague-Dawley	controls: 2 groups, 36 and 41; treated: 13-21/dose	corn oil	commercial grade	dose equimolar to 12.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	12.5	Increased ($p<0.05$) incidence of subcutaneous edema among fetuses; no effect on maternal body weight	Schwetz et al, 1971
					dose equimolar to 25 mg/kg/day 2,4-D on days 6 through 15 of gestation	25	Increased ($p<0.05$) incidence of sternebrae with split centers of ossification among litters and of delayed ossification of skull bones among fetuses; no effect on maternal body weight	
					dose equimolar to 50 mg/kg/day 2,4-D on days 6 through 15 of gestation	50	Intermediate response between 2 lower dosage levels (12.5 and 25 mg/kg/day) and 2 higher dosage levels (75 and 87.5 mg/kg/day)	
					doses equimolar to 75 or 87.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	75 or 87.5	Decreased ($p<0.05$) fetal weight among litters; increased ($p<0.05$) incidences of skeletal defects (including delayed ossification of sternebrae and/or skull, wavy ribs, lumbar ribs, missing sternebrae) among fetuses and litters; increased ($p<0.05$) incidence of subcutaneous edema among fetuses and litters; no effect on maternal body weight	

Compound	Species/ Strain	No. Dams at Start	Vehicle	Purity	Dosage/Exposure	Dose as mg/kg/day of 2,4-D	Maternotoxic, Fetotoxic and Teratogenic Effects	Reference
PGBE ester of 2,4-D	rats/CD	~37/group for vehicle controls and 2 lower dose groups; 28 at 25 mg/kg/day; 19 at 87.5 mg/kg/day	corn oil	97.15%	doses equimolar to 6.25, 12.5 or 25.0 mg/kg/day 2,4-D on days 6 through 15 of gestation	6.25, 12.5 or 25.0	No adverse effects on fetuses or dams	Unger et al., 1981
					dose equimolar to 87.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	87.5	"Minor embryo-toxicity which was not deleterious to growth and survival", i.e., statistically significantly increased incidence of lumbar (14th) rib buds; no adverse effects on body weight or survival of dams	
Isooctyl ester of 2,4-D	rats/CD	~35/group for vehicle controls and 2 lower dose groups; 28 at 25 mg/kg/day; 21 at 87.5 mg/kg/day	corn oil	96.6%	doses equimolar to 6.25, 12.5 or 25.0 mg/kg/day 2,4-D on days 6 through 15 of gestation	6.25, 12.5 or 25.0	No adverse effec or fetuses or dams	Unger et al., 1981
					dose equimolar to 87.5 mg/kg/day 2,4-D on days 6 through 15 of gestation	87.5	"Minor embryo-toxicity which was not deleterious to growth and survival", i.e., statistically significantly increased incidence of lumbar (14th) rib buds; no adverse effects on body weight or survival of dams	
2,4-D	rats/NR	NR	OP-7	NR	50 mg/kg/day 2,4-D on days 7 through 14 of gestation	50	Increased hemorrhages in the thoracic and abdominal cavities and in the liver and soft tissues of fetuses; details of maternal toxicity were not reported	Konstantinova et. al, 1976
2,4-D; three samples	golden Syrian hamsters	controls: 86; treated: 7-12/ dose/2,4-D sample	acetone, corn oil, carboxy-methyl cellulose	no dioxins detected	20, 40, 80 or 100 mg/kg/day 2,4-D on days 6 through 10 of gestation; 3 or 4 dose levels/ 2,4-D sample	20, 40, 80 or 100	Decreased ($p<0.05$) fetal viability among litters at 40 mg/kg/day with 1 of 3 samples and at 80 and 100 mg/kg/day with another sample; no other significant effects or malformations; details of maternal toxicity were not reported	Collins and Williams, 1971

*Khera and McKinley (1972) did not score minor growth retardation and delays in ossification as anomalous developmental patterns. Skeletal malformations included wavy ribs, additional ribs, fused ribs; retarded ossification of frontal and parietal bones; sternal defects; small distorted scapula laterally convex or distorted humerus shaft, and bent radius or ulna, resulting in micromelia of the forelimb.

NR = Not reported

No cleft palates were observed in control animals. In all high dose groups, fetal weight among litters was significantly decreased ($p<0.05$). The incidence of cleft palate in offspring of dams treated with 2,4-D was 2 and 6% cleft palate/litter for the low and high doses, respectively. In both groups of animals treated with 2,4-D, no effect on maternal weight gain was noted, but significant increases ($p<0.05$) in maternal relative liver weight were observed. A NOAEL for the n-butyl ester derivative may be defined at 124 mg/kg/day, as no effect on fetal parameters or on maternal weight gain or relative liver weight was observed at this dose level. At the high dose, the n-butyl ester induced 15% cleft palate/litter; a significant increase ($p<0.05$) in maternal relative liver weight without any effect on maternal weight gain was also observed. The PGBE ester of 2,4-D was the most toxic compound tested, inducing 8 and 16% cleft palate/litter at 124 and 221 mg/kg/day, respectively. In addition, treatment with 124 mg/kg/day of the PGBE ester resulted in significantly decreased ($p<0.05$) fetal weight among litters and significantly increased ($p<0.05$) maternal relative liver weight. No incidence of cleft palate or maternal toxicity was observed in animals treated with 124 mg/kg/day of either the isopropyl or isooctyl ester; fetal weight among litters was significantly decreased ($p<0.05$). 2,4-D butyric acid, administered at 124 mg/kg/day, did not induce cleft palate but significantly increased ($p<0.05$) maternal relative liver weight. Based on a calculated prenatal development index value that considered both fetotoxicity and developmental effects (cleft palate), Courtney (1977) observed that the relative order of prenatal toxicity of these compounds was PGBE ester > 2,4-D > isopropyl ester > isooctyl ester > n-butyl ester for the low level of compounds administered on days 7 through 15 of gestation.

Khera and McKinley (1972) studied the teratogenic and postnatal effects of 2,4-D; the isooctyl, butyl, and butoxyethanol esters of 2,4-D; and the dimethylamine salt of 2,4-D administered orally to rats on days 6 through 15 of gestation. Day 1 of gestation was designated as the day after mating for females having sperm in a vaginal smear; the dams were killed on day 22 of gestation. Three commercial preparations and one purified (recrystallized) preparation of 2,4-D (no dioxins detected) were tested at two or more dose levels of 25, 50, 100 and 150 mg/kg/day. The ester derivatives were administered at 50 and 150 mg/kg/day, while the dimethylamine salt derivative was tested at 100 and 300 mg/kg/day. Appropriate vehicle (corn oil or aqueous gelatin) controls were used for each compound tested. Neither administration of 2,4-D nor any of its derivatives had an effect on maternal body weight. For the purposes of this study, minor growth retardation and delayed ossification were not classified as teratogenic effects by the authors. Skeletal malformations included wavy ribs, additional ribs, fused ribs; retarded ossification of frontal and parietal bones; sternal defects; and small, distorted scapula, laterally convex or distorted humerus shaft, and bent radius or ulna, resulting in micromelia of the forelimb. 2,4-D induced a significantly increased incidence ($p<0.05$) of skeletal malformations at 25, 100 and 150 mg/kg/day; the incidence at 50 mg/kg/day was higher than in controls but was not statistically significant. The authors expressed reservations about the significance obtained at the 25 mg/kg/day level, but two of the fetuses from one of two replicate groups treated with this dosage had malformations of the forelimb (previously described). Such malformations were not observed in any control fetuses. A dose-related increase in fetal mortality and a decrease in fetal weight were also significant ($p<0.05$) at the two highest dose levels of 2,4-D. A significantly increased incidence ($p<0.05$) of skeletal malformations was observed in

offspring of dams fed 150 mg/kg/day of the isooctyl, butyl, or butoxyethanol ester of 2,4-D or 300 mg/kg/day of the 2,4-D dimethylamine salt. Fetal body weights were depressed at the 150 mg/kg/day level of the isooctyl and butyl esters. A NOAEL for the isooctyl, butyl, and butoxyethanol esters may be defined at 50 mg/kg/day, and for the dimethylamine salt at 100 mg/kg/day, as judged by no apparent adverse effect on fetal mortality, fetal weights or occurrence of skeletal or visceral anomalies. Postnatal survival was not affected at levels up to (but not including) 200 mg/kg/day of 2,4-D, 150 mg/kg/day of the ester derivatives or 300 mg/kg/day of the salt derivative, leading the authors to conclude that the observed skeletal defects were not incompatible with survival of newborn pups.

Teratogenic effects following oral administration of 2,4-D and the isooctyl and PGBE esters of 2,4-D to Sprague-Dawley rats were investigated in a three-part study by Schwetz et al. (1971). In the first part of the study, pregnant rats were treated with commercial grades of compound in corn oil suspension or solution at levels of 12.5, 25.0, 50.0, 75.0 or 87.5 mg 2,4-D/kg/day or molar equivalents of the esters on days 6 through 15 of gestation. The day sperm were first observed in a vaginal smear was considered to be day 0 of gestation; dams were killed on day 20 of gestation. The maximum tolerated dose of 2,4-D was found to be 87.5 mg/kg/day for Sprague-Dawley rats. Control animals were administered 2.5 mL corn oil/kg/day orally. No effect on maternal body weight was observed with any of the three compounds at the levels tested. At the 12.5 mg 2,4-D/kg/day dose level, treatment with 2,4-D resulted in a significantly increased incidence ($p<0.05$) of delayed ossification of skull bones among fetuses and litters, the PGBE ester resulted in a significantly increased incidence ($p<0.05$) of missing sternebrae among fetuses; and the isooctyl ester resulted in a

significantly increased incidence ($p<0.05$) of subcutaneous edema among fetuses. The NOAEL in Wistar rats of 50 mg/kg/day of isooctyl ester (equivalent to 33.2 mg/kg/day of 2,4-D) derived from the Khera and McKinley (1972) study may be indicative of strain differences, as a LOAEL of 12.5 mg/kg/day of 2,4-D as the isooctyl ester may be inferred from this study for Sprague-Dawley rats. At the 25 mg 2,4-D/kg/day level, treatment with 2,4-D had no effect on fetuses; treatment with the PGBE ester resulted in a significantly increased incidence ($p<0.05$) of delayed ossification of skull bones among fetuses and litters; and the isooctyl ester resulted in significantly increased incidences ($p<0.05$) of sternebrae with split centers of ossification among litters and of delayed ossification of skull bones among fetuses. Schwetz et al. (1971) pointed out that the incidences of these effects varied considerably between the two vehicle control groups. Treatment with 50 mg/kg/day of 2,4-D on equimolar doses of the esters gave an intermediate response between the two lower dosage levels (12.5 and 25 mg/kg/day) and the two higher dosage levels (75 and 87.5 mg/kg/day) for all three compounds tested. Treatment with 75 or 87.5 mg/kg/day of 2,4-D, or equimolar doses of PGBE ester, or the isooctyl ester yielded decreased fetal weight among litters, significantly increased incidences ($p<0.05$) of skeletal defects (including delayed ossification of sternebrae or skull (or both), wavy ribs, lumbar ribs and missing sternebrae) among fetuses and litters, and a significantly increased incidence ($p<0.05$) of subcutaneous edema among fetuses and litters. Schwetz et al. (1971) stated that, in their opinion, the "dose level essentially without effect" was 25 mg 2,4-D/kg/day for 2,4-D and its PGBE and isooctyl esters. They classified all of the anomalies as embryotoxic or fetotoxic effects rather than as teratogenic responses, because none of these anomalies adversely affected either fetal or neonatal development and survival.

In the second part of the study, Schwetz et al. (1971) evaluated the effect on implantation of the PGBE and isooctyl esters of 2,4-D, administered orally on days 5 through 8 of gestation at levels constituting the molar equivalent of 87.5 mg 2,4-D/kg/day. Neither administration of the PGBE ester nor the isooctyl ester affected the percentage of pregnancies or the number of implantations. In the third part of the study, the isooctyl ester was administered orally at a level constituting the molar equivalent of 87.5 mg 2,4-D/kg/day on days 8 through 11 or on days 12 through 15 to differentiate between effects observed in early and late organogenesis. An increased incidence of resorptions was seen in early but not late organogenesis. Isooctyl ester had no effect on fetal body measurements during either stage of organogenesis. The incidence and magnitude of sternebral anomalies was similar following treatment with isooctyl ester during either stage of organogenesis; however, subcutaneous edema was observed only in fetuses of dams treated during early organogenesis.

In a study similar to the first part of the Schwetz et al. (1971) study, Unger et al. (1981) investigated the teratogenic and postnatal effects of the PGBE and isooctyl esters of 2,4-D administered orally to CD rats. Groups of pregnant rats were dosed with the PGBE or isooctyl ester at doses equivalent to 0, 6.25, 12.5, 25.0 or 87.5 mg 2,4-D/kg/day on days 6 through 15 of gestation. The day of detection of sperm in vaginal smears was taken as day 0 of gestation; dams were killed on day 20 except for those designated for a postnatal study. In the offspring of dams ingesting 87.5 mg 2,4-D/kg/day as the PGBE or isooctyl ester, minor fetotoxicity [statistically significantly increased incidences of lumbar (14th) rib buds] was observed; however, maternal toxicity, abnormal postnatal growth and development,

reduced fetal survival or teratogenic effects were not observed. Unlike the previous study of Schwetz et al. (1971), no adverse effects were produced at any of the lower dose levels of either ester.

Konstantinova et al. (1976) have studied the possible teratogenic effects of 2,4-D, 2,4-dichlorophenol and the combination of these two compounds given orally to rats. Compounds were administered intragastrically as aqueous emulsions in OP-7, a mixture of oxyethylated alkylphenols. Increased hemorrhages in the thoracic and abdominal cavities and in the liver and soft tissues were observed in fetuses taken from animals treated with 50 mg/kg/day 2,4-D on days 7 through 14 of gestation or 1 mg/kg/day 2,4-dichlorophenol on days 1 through 20 of gestation. No other gross anatomical effects were found, and increased embryolethality was not observed. The combination of 0.1 mg/kg/day 2,4-D and 0.1 mg/kg/day 2,4-dichlorophenol administered on days 7 through 14 of gestation also produced an increased hemorrhaging in internal organs. The authors expressed concern about the positive effect seen with the combination of 2,4-D and a metabolite (2,4-dichlorophenol) occurring at a level at which neither compound alone shows toxicity.

Aleksaskina et al. (1973) investigated the embryotoxicity of the diethylamine salt of 2,4-D administered orally to rats. Administration of 0.5 mg/kg of the compound throughout pregnancy produced decreases in fetal weight and length. When single doses of the 2,4-D salt were administered on day 4, 6, 9 or 13 of gestation at one-half the LD_{50} level (~400-600 mg/kg), an increase in fetal abdominal hemorrhages was observed. The nature of these lesions was not defined in the available abstract. This level of compound given orally on day 5, 9, 10 or 13 produced increased fetal deaths,

and on day 5, 9 or 13, produced decreased fetal weights. No information was available on the strain of animals tested, the purity of the compound, or the type of vehicle used. No skeletal examinations were conducted during this investigation.

Further work by this group (Buslovich et al., 1976) compared the embryotoxic effects of the diethylamine salt with those of the sodium salt, butyl ester, and amine salt of 2,4-D following oral administration to rats. Administration of the butyl ester at a single dose of one-half the LD_{50} value produced increased fetal deaths and resorptions when the compound was given as a single dose on day 4, 5, 6, 9, 10, 11 or 13 of gestation. The sodium and amine salts given at this dose did not produce embryolethal effects; however, when administered on either day 10 or 14 of gestation, they produced a decrease in fetal weight and length. The authors indicated that the butyl ester of 2,4-D produced the most severe embryotoxic effects, followed by the diethylamine salt, and then the sodium and amine salts. This conclusion appears to be based on comparisons of effects produced by one-half the LD_{50} level, which does not necessarily represent equitoxic doses. Complete data from this study were not available for review. The purity of the compounds and the vehicles used for administration were not identified in the available abstract.

The teratogenic potential of 2,4-D administered orally to hamsters was investigated by Collins and Williams (1971). Three commercial 2,4-D samples (no dioxins detected) were fed by intubation at levels of 20-100 mg/kg/day to hamsters on days 6 through 10 of gestation. The day after mating was designated day 0 of gestation; the dams were killed on day 14 of gestation.

Controls were fed the vehicle containing acetone, corn oil and carboxymethyl cellulose. No significant teratogenic or other effects were noted with any of the preparations. Decreased fetal viability was noted with feeding of one 2,4-D sample at 80 and 100 mg/kg/day doses, while another 2,4-D sample showed this effect at 40 mg/kg/day; the authors did not consider this a clear dose-related response.

Bionetics Research Laboratories (1968b) initiated a large-scale screening program in 1964 to investigate the teratogenic potential of a number of herbicides and other chemicals under a contract with the National Cancer Institute. Included in this study were 2,4-D, the butyl, isooctyl, isopropyl, methyl, and ethyl esters of 2,4-D, and the metabolite, 2,4-dichlorophenol. All compounds were tested by daily s.c. injections in several strains of mice, usually in doses of 46-150 mg compound/kg/day, in DMSO on days 6 through 14 of gestation (days 6-15 of gestation in AKR mice); dams were killed on day 18 (or day 19 for AKR mice). Day 0 of gestation was the day of detection of a vaginal plug. 2,4-D (100 mg/kg/day in 50% honey in water, 0.1 mℓ) was also administered by gavage. Fetotoxic and teratogenic effects were observed for certain groups of mice administered 2,4-D orally or s.c., and for the butyl, isooctyl, and isopropyl esters of 2,4-D, and for 2,4-dichlorophenol injected subcutaneously. These effects were generally seen at a dose of compound corresponding to 100 mg/kg/day of 2,4-D. The methyl and ethyl esters of 2,4-D produced some decreases in fetal weights at this level of administration but failed to produce teratogenic effects. The majority of teratogenic effects observed were in one strain of mice, BL-6, and included such defects as microthalmia, agnathia and anophthalmia. This strain of mice showed inconsistent responses to the administered compound; results with BL-6 mice were therefore divided to include those animals

treated with compounds from September through November, 1966, and those animals that were treated after November, 1966. Even responses of control BL-6 mice to s.c. injection with DMSO varied widely during these two time periods. Data on maternal toxicity were not presented for animals treated with the chlorophenoxy compounds. Because these studies were conducted as part of a large-scale screening program, studies using other doses of compounds were not generally done and, thus, dose-response relationships cannot be evaluated. The s.c. route of administration further complicates the interpretation of these results when applied to the more likely routes of exposure (inhalation and oral).

Teratogenic and embryotoxic effects have been observed in mice following s.c. injection of Hormoslyr 64, a commercial preparation that contains 2,4-D at 330 g/ℓ and 2,4,5-T at 170 g/ℓ (Bage et al., 1973). DMSO, used as a vehicle for test solutions, contained an unspecified mixture of petroleum distillates. Animals were injected on days 6 through 14 of pregnancy and killed on day 18. When this preparation was injected at a dose of 110 mg/kg, an increase was observed in fetal cleft palate incidence, and fetal weight and fetal survival were decreased. Injection of 50 mg/kg doses produced a small increase in cleft palate incidence and no significant embryotoxic effects. Other skeletal and internal malformations noted after administration of the high level of the 2,4-D/2,4,5-T mixture included increased rib and vertebral abnormalities and increased renal and subcutaneous hemorrhages. The authors noted that cystic kidneys and dilation of the renal pelvis, defects reported by other investigators after administration of 2,4,5-T to rats, were not seen in this investigation. As noted previously in the Bionetics (1968b) study, the significance of this route of adminis-

compounds from September through November, 1966, and those animals that were treated after November, 1966. Even responses of control BL-6 mice to s.c. injection with DMSO varied widely during these two time periods. Data on maternal toxicity were not presented for animals treated with the chlorophenoxy compounds. Because these studies were conducted as part of a large-sca . screening program, studies using other doses of compounds were not generally done and, thus, dose-response relationships cannot be evaluated. The s.c. route of administration further complicates the interpretation of these results when applied to the more likely routes of exposure (inhalation and oral).

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tration is questionable. Some malformations and hemorrhaging were observed in vehicle-treated animals; the complicating effect of DMSO is therefore added to the combination of the two herbicides.

Lutz-Ostertag and Lutz (1970) reported embryotoxic and teratogenic effects after spraying 2,4-D amine on pheasant and partridge eggs. A commercial preparation of the herbicide was sprayed at a level of 1.1 kg/ha after eggs had been incubated for 3.5 days. These investigators found high mortality (43-77%) of embryos by days 20-22 of development. Surviving embryos showed paralysis and sterility. Morphological examinations showed fused vertebrae, curled toes, and testicular and ovarian anomalies. The type of vehicle used and impurities present in the 2,4-D preparation were not described, nor were data on controls presented. No embryonic effects were found following spraying on chicken eggs at a level of 11.2 kg/ha with a commercial 2,4-D PGBE ester formulation (Somers et al., 1978), while immersion of chicken eggs in 1% solutions of commercial 2,4-D amine preparations has also been reported to produce no significant toxic effects (Gyrd-Hansen and Dalgaard-Mikkelsen, 1974). The findings of Lutz-Ostertag and Lutz (1970) appear to be unique and suggest an effect not attributable to the 2,4-D component. Gyrd-Hansen and Dalgaard-Mikkelsen (1974), however, did find increased mortality, malformed beak and gastroschisis in chick embryos following direct injection of the yolk sac with 2-10 mg/egg of commercial 2,4-D amine. The significance of embryonic effects produced by direct injection of chicken eggs for determining the teratogenic potential of chlorophenoxys is uncertain.

Other Reproductive Effects. Hansen et al. (1971) investigated the effects of long-term dietary administration of technical grade 2,4-D in a 3-generation reproduction study in rats. Starting at 3 weeks of age, rats were maintained on diets containing 100, 500 or 1500 ppm (~5-75 mg/kg bw) of 2,4-D for 2 years. Data from the F_1, F_2 and F_3 generations indicated an increase in preweanling mortality at the 1500 ppm feeding, determined as the percentage of pups weaned. At this maximum dose of compound, a significant loss in weight of weanlings was also observed, but there was no effect on parental fertility or litter size. No deleterious effect was noted at the lower dose levels (100 and 500 ppm) on fertility, mean litter size or viability of pups during the first 21 days of age. Liver aliesterase and liver acylamidase activity did not differ between selected F_2 rats (10/sex) at 90 days of age. These authors cite unpublished work by Gaines and Kimbrough (1970), who found increased mortality of weanlings following administration of 1000 or 2000 ppm 2,4-D to groups of 10 female rats in the diet for 3 months before mating and throughout pregnancy and lactation. At 2000 ppm, the pups were small at birth and 94% died before weaning. At 1000 ppm, more deaths were seen among offspring of treated dams than were seen among offspring of control dams.

Bjorklund and Erne (1966) observed toxic effects in the offspring of a single pig fed 500 mg/kg 2,4-D triethanolamine in the diet throughout pregnancy and for 6 weeks after delivery (~25-50 mg/kg/day). Underdevelopment and increased mortality were observed in newborn piglets in the first 24 hours after parturition. "Anorexia" was observed in the sow throughout the experiment; she died ~6 weeks after delivery.

As part of a two-generation study, Bjorklund and Erne (1966) administered 0 or 1000 ppm (equivalent to 0 or ~50-100 mg/kg/day, based on water consumption) of 2,4-D in the drinking water of pregnant rats (N=5/group) throughout gestation and for 10 months following parturition, and continued with offspring for up to 2 years after weaning. Although a reduced food and water intake, with consequent growth retardation, was observed in the treated offspring, no clinical signs, malformations or distinct morphological changes were seen in treated offspring. This study is also discussed in Chapter VII.

In a reproduction and fertility study, Lamb et al. (1980) administered a mixture of 2,4-D, 2,4,5-T and 2,3,7,8-TCDD (simulated Agent Orange) to male C57Bl/6 mice. Groups of animals were given feed that contained various concentrations of the three compounds, so that the daily doses of 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T, and 2.4 µg/kg 2,3,7,8-TCDD (Group II) or 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T, and 0.16 µg/kg 2,3,7,8-TCDD (Group III) or 20 mg/kg 2,4-D, 20 mg/kg 2,4,5-T, and 1.2 µg/kg 2,3,7,8-TCDD (Group IV) would be achieved. Another group of animals, used as controls (Group I), were given feed with only the corn oil (2%) vehicle added. At the end of 8 weeks, the treated animals were reported to have dose-related liver and thymus toxicity and significantly reduced body weight gain; however, the liver and thymus recovered to normal or near normal weights following termination of treatment. No significant effect was noted on sperm abnormalities either during or following the dosing period. After 8 weeks of treatment, each treated male was mated to three untreated virgin females, for a total of 24 matings for each treated male. Exposure to the various mixtures of simulated Agent Orange did not result in a significant decrement in fertility or reproduction, as evidenced by no effect on mating frequency, average

fertility, percent implantation and resorption sites, and percent fetal malformations. Furthermore, germ cell toxicity was not observed, and the offspring of treated males were not affected by increased lethality or abnormal neonatal development.

Summary

Acute exposure to high levels of 2,4-D results in progressive symptoms of muscular incoordination, lethargy, hindquarter paralysis, stupor, coma and death in animals (Hill and Carlisle, 1947). These symptoms have been observed consistently in a variety of species regardless of route of administration, and myotonia appears to be a dominant effect of exposure (Bucher, 1946; Hill and Carlisle, 1947; Drill and Hiratzka, 1953). Pathological examinations have shown that acute exposure to high levels of 2,4-D resulted in kidney damage and skeletal muscle changes in a variety of species, but hepatic damage has been described only in dogs (Hill and Carlisle, 1947; Drill and Hiratzka, 1953). Acute oral LD_{50}s in the range of 350-500 mg/kg 2,4-D have been reported for rodents, but significant differences in toxicity are not apparent between 2,4-D and its salts and esters. Inhalation toxicity data are not available.

Subchronic oral toxicity studies have been conducted with rats that were exposed to 2,4-D at levels of 3-300 mg/kg/day, 5 days/week for 4 weeks (Rowe and Hymas, 1954), 2000 ppm in the diet for 4-7 weeks (Chang et al., 1974); 100-5000 ppm in the diet for 113 days (Rowe and Hymas, 1954) and 200-400 ppm in the diet for 31 days (Hill and Carlisle, 1947). In rats doses of 5.0 mg/kg bw/day or higher resulted in significant reductions in blood indices at all doses; liver enzyme activities were reduced at higher doses; kidney toxicity was also evident at higher doses. Effects of higher doses included

GI irritation and mild liver effects (e.g., cloudy swelling, increased weights), as well as characteristic overt signs of toxicity and mortality. In mice doses of 15.0 mg/kg bw/day or higher are associated with decreases in brain weight and increases in white blood cells and pituitary gland hypertrophy as well as other effects (Hazelton Laboratories, 1983). Treatment-related signs of intoxication or mortality were not observed in guinea pigs exposed to 10 daily 50 or 100 mg doses of 2,4-D sodium salt by intubation in 12 days (~88 or 177 mg/kg/day 2,4-D acid equivalent) (Hill and Carlisle, 1947). Administration of 20 mg/kg/day 2,4-D in capsules 5 days/week for 13 weeks produced some mortality but not significant lesions in dogs, but similar exposure to <10 mg/kg/day produced no evidence of toxicity (Drill and Hiratzka, 1953). Pathological and functional effects have been described in the liver, kidneys, lungs, thyroid and nervous system of rats and mice that were repeatedly injected (subcutaneously or intraperitoneally) with 100-200 mg/kg/day 2,4-D (Bucher, 1946; Florsheim and Velcoff, 1962; Florsheim et al., 1963; Desi et al., 1962). Systemic toxicity was not produced by the daily (5 days/week) application of 2,4-D dimethylamine salt, isooctyl ester or butyl ester to the skin of rabbits for 3 weeks at levels of 2,4-D acid corresponding to 0.6 and 3.1% (Kay et al., 1965).

Significant treatment-related gross, histopathological or hematological effects were not found in rats that were exposed to 5-1250 ppm levels of 2,4-D in the diet (~0.25-62.5 mg/kg bw/day) for 2 years (Hansen et al., 1971). In a 2-generation study, administration of 1000 ppm 2,4-D in the drinking water for up to 2 years had no effect on clinical chemistry indices or tissue histology in maternal rats or in the first or second generation offspring (Bjorklund and Erne, 1966). Treatment-related effects were not observed in dogs that were fed 10-500 ppm 2,4-D in the diet (~0.5-25

mg/kg/day) (Hansen et al., 1971). EEG changes were reported in monkeys that were exposed orally to 0.2 mg/kg/day 2,4-D for 3 years, but the toxicological significance of the changes is unknown (Santolucito, 1975).

Administration of 2,4-D to mice by intubation from days 7-28 of age at a level of 100 mg/kg initial bw and subsequently in the diet at a level of 323 ppm (~42 mg/kg bw/day) for 81-90 weeks was not tumorigenic (Bionetics Research Lab., 1968a; Innes et al., 1969). Similar administration of 2,4-D isopropyl ester, butyl ester or isooctyl ester [46.4 mg/kg by intubation, 111-130 ppm in the diet (~14.4-16.9 mg/kg bw/day) for the subsequent 73-83 weeks] was also nontumorigenic. Hansen et al. (1971) reported that administration of 2,4-D at levels of 5-1250 ppm in the diet (~0.25-62.5 mg/kg bw/day) for 2 years did not induce treatment-related tumors in rats, but reexamination of the histopathology sections by Reuber (1979) found a significant increase in the incidence of lymphosarcomas in females at all dose levels; the differences in tumor incidences have not been resolved. Rats or mice that were fed 2,4-D amine salt at one-tenth the LD_{50} level for life did not develop a significant increase in tumors (Archipov and Kozlova, 1974). Single s.c. injections of 2,4-D (215 or 464 mg/kg), 2,4-D isopropyl ester (100 mg/kg) or 2,4-D isobutyl ester (21.5 mg/kg) at age 28 days were not tumorigenic to mice after 78 weeks, but similar injection of 2,4-D isooctyl ester (21.5 mg/kg) induced a statistically significant increase in reticulum cell carcinomas (Bionetics Research Lab., 1968a). Repeated dermal application of 2,4-D reportedly produced skin papillomas in mice only when 2,4-D treatment was preceded by a single dermal application of the initiator 3-methylcholanthrene (Archipov and Kozlova, 1974). A rat bioassay (Industry Task Force, 1985) is not evaluated in this assessment. Note is made that EPA's Office of Pesticide Programs report tumor elevation at the high dose

in male rats and a marginally significant dose-response trend in the males. The female rats and mice were negative. Taken as a whole the animal evidence has been proposed by the Office of Pesticides (EPA 1988, Federal Register) to be inadequate.

2,4-D has not produced mutagenic effects in assays with Salmonella or bacteriophage T_4, although positive responses were reported by Simmon (1979) for DNA repair-deficient strains of E. coli and B. subtilis. Gene conversion/combination tests with the yeast, S. cerevisiae, were positive only when the pH of the system was lowered into the acid range (Siebert and Lemperle, 1974; Zetterberg et al., 1977; Zetterberg, 1978), where 2,4-D would be nonionized. 2,4-D was weakly mutagenic for D. melanogaster in recessive lethal and somatic mutation assays (Magnusson et al., 1977; Rasmusson and Svahlin, 1978). 2,4-D induced ouabain resistance in Chinese hamster V-79 lung cells (Ahmed et al., 1977), induced unscheduled DNA synthesis in cultured human fibroblasts (Ahmed et al., 1977), and induced chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes (Pilinskaya et al., 1976; Korte and Jalal, 1982), but was inactive in other in vitro mammalian assays (cell transformation in human lung or hamster kidney cells, unscheduled DNA synthesis in rat hepatocytes, chromosome aberrations in embryonic bovine kidney cells). Intraperitoneal injection of 2,4-D induced bone marrow chromosome aberrations (Pilinskaya et al., 1976) and oral administration of 2,4-D inhibited thymidine incorporation into testicular DNA in mice (Seiler, 1979), but other in vivo mammalian assays with mice (micronucleus assay and dominant lethal assay) were negative. Information regarding the mutagenicity of 2,4-D esters in animal systems was not located, but these compounds would theoretically penetrate cells more readily than 2,4-D at physiological pH.

Teratogenicity testing has been conducted with 2,4-D, several of its esters (n-butyl, isopropyl, isoctyl, PGBE, butoxyethanol), the dimethylamine salt, and 2,4-D butyric acid in mice, rats and hamsters (Courtney, 1977; Khera and McKinley, 1972; Schwetz et al., 1971; Unger et al., 1981; Konstantinova et al., 1976; Collins and Williams, 1971). Overall, these studies indicate that 2,4-D and its derivatives are embryotoxic but only weakly teratogenic or nonteratogenic. Oral doses (expressed as 2,4-D) of 124 mg/kg/day in mice (Courtney, 1977), 75-125.5 mg/kg/day in rats (Schwetz et al., 1971; Unger et al., 1981; Khera and McKinley, 1972) and 40-100 mg/kg/day in hamsters (Collins and Williams, 1971) produced fetotoxic effects or malformations (cleft palates and other skeletal malformations). Increased preweanling mortality and weight loss were observed in the offspring of rats that were exposed to 1500 ppm levels of 2,4-D in the diet in a 3-generation reproduction study, but adverse effects on litter size or fertility were not found (Hansen et al., 1971).

VI. HEALTH EFFECTS IN HUMANS

Acute Effects

Clinical reports have described fatal poisoning in humans resulting from ingestion of 2,4-D solutions. Nielsen et al. (1965) described a suicide case involving a male agricultural student who ingested at least 6 g of a commercial herbicide preparation of the dimethylamine salt of 2,4-D (50%, by weight). Death appeared to have been preceded by vomiting and convulsions. Pathological examination revealed acute congestion in most orgars as well as acute pulmonary edema, and histological examination showed degenerative ganglion cell changes in the brain. Geldmacher et al. (1966) reported two cases of poisoning with 2,4-D. Vomiting and loss of consciousress occurred in a 33-year-old woman following ingestion of an unknown quantity of 2,4-D. Terminal symptoms developed after 1 day, and included weak pulse, tachycardia and deep breathing. These authors described another case reported by Herbich and Machata (1963) of a 46-year-old man who died within 14 hours of swallowing at least 13.5 g of an uncharacterized 2,4-D solution. Constricted pupils and respiratory paralysis were noted among the terminal symptoms. At autopsy, both patients had generalized hyperemia of the organs as well as edema of the brain and the lungs. Dudley and Thapar (1972) reported the fatal poisoning of a 76-year-old male who ingested (assumedly) a pint of 2,4-D solution (in kerosene). Early symptoms included vomiting and loss of consciousness. Death occurred 6 days after the ingestion of 2,4-D. During medical treatment, the patient received pentobarbital, ampicillin and quinidine. Pathological examination indicated edema of the lungs, mid-zonal hepatic necrosis and pyelonephritis. The pulmonary effects noted may have been due to the kerosene vehicle. Multifocal perivascular plaques of demyelinization were observed during microscopic examination of the brain. The

authors attributed death to atrial fibrillation; however, in this case, as in the other fatal poisonings described, the exact cause of death is unknown.

Berwick (1970) reported an incident of nonfatal poisoning, in which a farmer swallowed a mouthful of concentrated weed killer containing 2,4-D. Initial symptoms included acute gastritis and vomiting. Eighteen hours later, intense aching of the chest, painful and tender muscles, and a tender abdomen were reported. Within the next 6 hours, the patient lost use of his intercostal muscles, and the muscles of his upper extremities exhibited spontaneous fibrillary twitchings. Hyperactive biceps and triceps reflexes were seen, but other reflexes were normal. The blood levels of several enzymes, including lactate dehydrogenase, SGOT, SGPT, aldolase and creatine phosphokinase, were increased from days 4-7; myoglobinuria was observed, indicating probable skeletal muscle damage. Although this commercial formulation of weed killer contained 49% Eptam, 35.5% 2,4-D isooctyl ester, 0.5% epichlorohydrin and 5% emulsifiers, the authors found no evidence that the Eptam produced toxicological effects or cholinesterase inhibition. Loss of sexual potency was observed in this patient and persisted for ~4 months; however, other symptoms subsided within ~2 weeks.

Neurological symptoms have been described in other reports of illness related to human exposure to 2,4-D. In most of the cases of occupational exposure to 2,4-D, the subjects had previously been exposed to a variety of pesticides; however, the neurological symptoms observed in 2,4-D exposed experimental animals (see Acute Toxicity Section in Chapter V) suggest that the compound has the potential to produce neurological effects. Monarca and DiVito (1961) reported symptoms in a farmer who became ill after applying a

40% aqueous solution of 2,4-D against the wind. The symptoms included ataxia, reflex disorders such as abolished Achilles tendon reflex and reduced patellary reflexes, and a positive Romberg's sign (damage to the dorsal column of the spinal cord). Symptoms persisted for 2-3 months and subsided slowly. Goldstein et al. (1959) described three cases of peripheral neuropathy following exposure to an ester of 2,4-D. The first patient had two dermal exposures within 2 months to spills of a 10% solution of 2,4-D ester, and experienced nausea, vomiting and diarrhea after each exposure. He experienced numbness and aching of the digits 1 week after the second exposure. During the following 5 weeks, he showed the development of peripheral neuropathy. The second patient also developed neurological symptoms after two exposures, ~1 year apart, to dermal wetting with an ester of 2,4-D. This patient developed flaccid paraparesis 5 months after the second exposure. The third patient reported wetting of his clothes with a spray solution of 2,4-D ester during application. Within 24 hours, he developed malaise, headache, nausea and vomiting, and, within 48 hours, severe vertigo. Paresthesia in the limbs appeared within 4-5 days, followed by fasiculations that became generalized. In all three cases, the symptoms of peripheral neuropathy were of prolonged duration. The authors obtained the label from one container that had been used for spraying and found that the applied material was 44% isopropyl ester and 56% inert ingredients. Another clinical report of long-term (~2 years) peripheral neuropathy in a farmer exposed to 2,4-D ester while spraying has been reported by Todd (1962). Berkley and Magee (1963) described what appeared to be a primary sensory neuropathy of the upper extremities in a farmer exposed to the

dimethylamine salt of 2,4-D while spraying a corn field. Bordas et al. (1958) also noted weight loss and sensory and motor neuropathies in workers exposed to 2,4-D during spraying operations.

Polyneuritis has been described in a farmer who became ill after spraying 2,4-D solutions of 235 and 410 g/l for several days in an open cab tractor (Foissac-Gegoux et al., 1962). The patient developed facial anaesthesia and paresthesia. He subsequently lost feeling in his legs and was forced to walk with a cane. Neurological examination showed increased knee reflex and decreased Achilles tendon reflexes at 2 months; 3 months after exposure, motor and sensory symptoms of the face and legs had improved, but an electromyelogram still showed abnormalities. In these reports of agricultural exposures, the precise formulations of compounds used is not known, nor is the amount of compound present in the air or on the skin of workers.

Subchronic and Chronic Effects

Noncarcinogenic Effects. Reports on subjective clinical symptoms in workers exposed to 2,4-D during its manufacture or use have been published. Assouly (1951) reported that symptoms in workers employed in the fabrication of 2,4-D esters included gastralgia, anorexia, somnolence, a sweet taste in the mouth, increased hearing sensitivity, a sensation of drunkenness and heaviness of the legs. Bashirov (1969) examined 292 workers (248 men and 44 women) engaged in the manufacture of 2,4-D amine and butyl ester. He reported a high frequency (63%) of symptoms of rapid fatigue, weakness, headache or vertigo. Approximately 20% of these workers experienced hypotension, bradycardia, dyspepsia and gastritis. Another Russian study (Fetisov, 1966) reported rapid fatigue, headache, loss of appetite, pains in

the region of the liver and stomach and lowered acuity of taste and smell in workers using preparations of the butyl ester, crotyl ester or amine salt of 2,4-D. Health effects of exposure to chlorophenoxys in a 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) production plant were reported by Poland et al. (1971). A study of health records included 73 male workers with an average duration of employment of 8.3 years. Symptoms reported for these workers included chloracne, hyperpigmentation and hirsutism; these correlated together and were most probably related to exposure to 2,4,5-T and dioxin impurities. Other symptoms noted included various gastro-intestinal disturbances and decreased hearing acuity. One case reported diminished proprioception and two workers failed to demonstrate ankle jerk reflexes. None of these subjective clinical reports indicate levels of exposure, nor do they indicate other possible chemicals to which these workers may have been exposed.

Kephart (1945) reported that an individual who voluntarily ingested 500 mg of 2,4-D daily for 21 days demonstrated no ill effects. Additional information regarding this observation is not available.

Wallis et al. (1970) described neurological changes in a worker exposed to 2,4-D over a period of 1 year while spraying sugar cane fields. Over a period of 2 days, this worker developed painful paresthesias in the hands and feet. During the next 3 days, he developed painful muscular stiffness in all four limbs; this condition progressed over the next 2 years, impairing his gait and his manual dexterity. Movement was with deliberate slowness and great effort. Medical evaluation showed fasiculations of facial, masseter, trunk and extremity muscles. Electromyography indicated normal-

appearing motor units and periodic outbursts of repetitive short durations. A biopsy of the right sural nerve showed that some fibers had undergone degenerative changes. No detectable 2,4-D was found in a urine sample from the patient. Treatment with diphenylhydantoin relieved the muscular rigidity in this patient as long as blood levels of drug were maintained.

Seabury (1963) administered a total of 12,712 mg of 2,4-D sodium salt, 369 mg of indole-3-butyric acid and 38.3 mg of α-naphthalene acetic acid to a patient that had terminal coccidioidomycosis during a period of 33 days without observable toxicity. The 2,4-D salt was administered daily in a total of 23 doses; four of the first five doses were given via intramuscular injection (8-24 mg/dose), doses 6 through 21 were given intravenously (doses 11-12 were 960 mg/dose and doses 13-21 were 800 mg/dose), and dose 22 was 2000 mg. Although administration of the 2,4-D until this point had been without apparent adverse effects, a final intravenous dose of 3600 mg 2,4-D sodium salt 2 days after the 2000 mg dose, infused over a 2-hour period, elicited a lapse into a semistuporous state, fibrillary muscle movements in the mouth and both hands that persisted for several hours, and hyporeflexia in the knees, ankles and biceps that persisted for 24 hours. The patient still complained of profound muscle weakness 24 hours after the infusion. Within the next 24 hours, recovery was observed, and no subsequent abnormalities in neurological or muscular parameters were noted in the following 2 weeks.

Johnson (1971) summarized an unpublished Dow Chemical study on the health effects of exposure to 2,4-D in a production facility. In this study, 220 men exposed for 0.5-22 years to 2,4-D in a range of 30-40 mg/day

were reported to show no significant clinical effects when compared with a control population of 4600 men not engaged in 2,4-D or 2,4,5-T manufacture. A battery of "at least 20 laboratory tests" was conducted, but additional details of this unpublished study were not presented. The author noted that karyotypes of peripheral blood lymphocytes from 10 of these workers showed no chromosome aberrations.

Singer et al. (1982) assessed the nerve conduction velocities of the median motor, median sensory and sural nerves of 56 employees (mean age of 35 years) engaged in the manufacture of 2,4,5-T and 2,4-D (exposure levels not determined) for an average of 7 years. The control group consisted of 25 subjects without previous exposure to neurotoxic agents, history of diabetes, stroke, other neurological disease or excessive alcohol use. Slowed nerve conduction velocities in one or more of the three tested nerves were seen in 46% of the study group, as compared with 5% of the control group. The most dramatic change occurred with sural sensory velocity, which was significantly correlated with duration of employment. The significance of these findings in relation to 2,4-D exposure to humans was not determined. Singer et al. (1982) indicated that another study of a group of workers exposed to 2,4,5-T contaminated with dioxins but not exposed to 2,4-D has been initiated. Comparison of these two studies may shed some light on the adverse effects in humans resulting from chronic exposure to 2,4-D.

Carcinogenic Effects.* Several Swedish epidemiological studies of workers exposed to chlorophenoxyacetic acids and derivatives have been published (Axelson and Sundell, 1974, 1977; Axelson et al., 1979; Hardell, 1977; Hardell et al., 1979, 1980; Hardell and Sandstrom, 1979; Eriksson et al., 1977). Axelson and Sundell (1974) studied tumor incidence and mortality in 730 Swedish railroad workers exposed for at least 45 days to various herbicides including chlorophenoxys, aminotriazole and monuron during spraying operations. Exposed workers were divided into four cohorts, based on the type of herbicide exposure, and mortality and tumor incidence were compared with national average age and sex specific values. Mortality was reported in the following categories: 1) all causes, 2) all tumors, and 3) lung cancers. Latency periods of 0, 3 and 5 years were included in calculations (i.e., workers exposed within the last 3 or 5 years were excluded from calculations). No excess for any of these three mortality causes was found for the cohort exposed to chlorophenoxys and combinations. Data from this study were recombined and reanalyzed by Axelson and Sundell (1977) to eliminate effects from combined exposure to chlorophenoxys and other agents (primarily aminotriazole). These investigators concluded that an excess tumor incidence can be positively associated with chlorophenoxy exposure alone, or with aminotriazole alone, and that the combination of the two herbicide types may potentiate the tumorigenic effect.

Axelson et al. (1979) considered this same group of railroad workers in a new study and extended the observation period from 1972 through October, 1978. Workers were divided into three cohorts: those exposed only to

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*For additional indepth analysis of the cancer epidemiology studies for phenoxy acetic acids and chlorinated phenols see U.S. EPA (1984).

aminotriazole, those exposed only to chlorophenoxys, and those exposed to both herbicide classes. Mortality evaluation included the following: 1) total mortality, 2) mortality produced by all tumors, 3) mortality produced by tumors of the stomach, or 4) mortality produced by tumors of the lungs. When a 10-year latency period was used in calculations, the cohort with combined herbicide exposure showed excesses in total mortality and mortality produced by all tumors (7 cases vs. 1.78 expected). Those workers who were exposed to chlorophenoxys showed only a small but significant excess of tumors of the stomach (2 vs. 0.33 expected, $p<0.05$). This excess in observed mortalities was associated with exposure to herbicides before 1962. The authors suggest that either the formulations used in that period were more toxic, or that work practices may have been more lax during the earliest period covered by this study (1957-1962). No estimates of the levels of exposure to chlorophenoxys or the duration of these exposures could be made. The 2,3,7,8-TCDD content of 2,4,5-T used by these workers was not determined. This extremely toxic contaminant may produce effects in the microgram range.

The discovery that 7 of 87 patients diagnosed with malignant mesenchymal tumors had a history of exposure to chlorophenoxy herbicides 10-20 years earlier led Hardell (1977) to conduct a case-referent study of 52 cases of soft-tissue sarcoma (STS) treated at Umea, Sweden, from 1970-1977 (Hardell and Sandstrom, 1979). The cases represented 21 living and 31 deceased male patients, ranging in age from 26-80 years. Control subjects were matched for sex, age, place of residence, and vital status. Deceased cases and controls were matched for year of death. Cohorts were defined as those exposed to chlorophenoxys only, those exposed to chlorophenols only, and

those exposed to both classes of compounds. Exposure information was obtained by questionnaires and supplementary interviews. These investigators calculated a relative risk of 5.3 (i.e., 5.3 x greater than the control risk) for developing STS in workers exposed to chlorophenoxys. This cohort had been exposed primarily to 2,4,5-T, 2,4-D and MCPA. The authors noted that 2,3,7,8-TCDD contamination of 2,4,5-T may have led to significant exposure. The number of patients with exposure to only chlorophenoxys used for the relative risk calculation was small (13 cases).

Another case-referent study on malignant mesenchymal tumors was initiated by this group for workers in a southern farming area of Sweden (Eriksson et al., 1977). Cases of STS were taken from the Swedish Cancer Registry of reports made from 1974-1978. Patients were males, 72 living and 38 deceased, ranging in age from 25-75 years. The design of the earlier study was retained for this investigation (i.e., two control subjects were selected for each case) and exposed workers were divided into three cohorts. The workers exposed to chlorophenoxys only showed a relative risk of 6.8 for developing STS. Workers exposed to phenoxy herbicides not known to be contaminated with polychlorinated dibenzo-p-dioxins (MCPA, 2,4-D, mecoprop, dichloroprop) showed a relative risk of 4.2. This subgroup, however, represented only 7 of the 25 cases of sarcoma considered by Eriksson et al. (1977). The authors suggest that this increased risk found in workers exposed to "nondioxin" herbicides alone could indicate carcinogenic effects produced by the chlorophenoxy herbicides themselves; however, materials to which this group was exposed were not available for analysis. In a later update of this work by Eriksson et al. (1981), he reiterated his earlier finding of a roughly 6-fold increase in the risk of STS from exposure to phenoxy acids or chlorophenols. Again, he found that the risk

ratio given exposure to phenoxy acids free of 2,3,7,8-TCDD and dibenzofurans was 4.2. When consideration was given to persons exposed only to phenoxy acids that contain such impurities, the relative risk equalled 17.0 This case-referent study was similar to that of Hardell and Sandstrom (1979) and subject to the same critisims. Recent work by Cochrane et al. (1980) indicates that 2,4-D may be contaminated with a small amount of chlorinated dibenzo-p-dioxins other than the 2,3,7,8-isomer.

Hardell et al. (1980) conducted a fourth case-referent study correlating the incidence of malignant lymphoma (both Hodgkin's and non-Hodgkin's lymphomas) with exposure to herbicides. The cases involved were 107 living and 62 deceased male patients treated in Umea, Sweden, between 1974 and 1978. The ages of patients ranged from 25-85 years. Workers exposed to chlorophenoxys alone showed a relative risk ratio of 4.8 for developing malignant lymphomas. The nature of the herbicides included in the category "chlorophenoxys" was not known, but probably included 2,4-D, 2,4,5-T, MCPA, picloram and aminotriazole (these latter two are not chlorophenoxy herbicides). Of the 41 cases of malignant lymphoma with histories of exposure to chlorophenoxys, seven cases were considered to have had primarily 2,4-D exposure. The authors suggest that by dividing the 41 lymphoma cases into two groups, based on 90 days or more estimated exposure to chlorophenoxys, an increase in relative risk (7.0 versus 4.3) is seen with a longer duration of exposure; however, this difference is not statistically significant. Methodology used in this study was the same as that used in the two earlier case-referent studies conducted by this group. Because exposure was determined on the basis of questionnaires and telephone interviews, very little detail on the nature and duration of exposure could be determined.

No increase in total mortality or deaths caused by malignant neoplasms was found by Ott et al. (1980) in a study of 204 employees engaged in the production of 2,4,5-T. Workers included in the study had been employed for 1 or more months between 1950 and 1971 in 1 of 4 jobs that involved exposure to chlorophenoxys and probably other agents such as chlorophenols and styrene-butadiene latex. An industrial hygiene survey conducted in 1969 found airborne exposure estimates of 0.2-0.8 mg/m³ for 2,4,5-T; <0.4 mg/m³ for 2,4-D; and 1.6-9.7 mg/m³ for 2,4,5-trichlorophenol. These levels, however, reflect only a single monitoring study and may have little relationship to levels 10-15 years earlier. Comparisons of worker mortality with average values for the total United States white male population using 5-year age intervals found no significant differences for the exposed worker population. The majority of the workers considered in this study had occupational exposure of <12 months total to chlorophenoxys.

Lynge (1985), in an incidence study of 3,390 males employed in two factories manufacturing phenoxyacetic acid herbicides, chiefly 2,4-D and MCPA, found a nonsignificant excess risk of STS in male employees. The author stated that these results supported the Swedish observation of an increased risk of STS following exposure to phenoxyacetic acid herbicides, including 2,4-D, "unlikely to be contaminated with 2,3,7,8-TCDD." However, after a 10-year latency, the excess of STS was significant (4 observed vs. 1.00 expected; $p<0.05$) in male employees of the single factory where 2,4,5-T had been produced and used and where all five STS's arose. However, the author cautions that because of the limited amount of 2,4,5-T processed at that factory exposure is unlikely although not impossible. The Lynge (1985) study noted only a slight excess of lymphomas in males after a lapse of 10

years from initial exposure (4 observed vs. 3.04 expected). However, sensitivity was somewhat reduced.

Hoar et al. (1986), in a population based case-referent study found significantly high rates of non-Hodgkin's lymphoma (NHL) in farmers in Kansas who use herbicides, particularly 2,4-D and triazines.

All newly diagnosed cases of STS, Hodgkins disease (HD) and non-Hodgkins lymphoma between 1976 and 1982 among white male residents were included in the study identified from the University of Kansas Cancer data service, a population-based registry for the state of Kansas. There were 200 diagnosed with STS, 173 diagnosed with HD and a random sample of 200 out of 297 men diagnosed with NHL during the period 1979 through 1981. (The period of time for the selection of STS and HD exterded from 1976 to 1982).

Three (3) white male controls (N=1005) were matched to each patient on age ($\pm$2 years) and vital status. Live controls over 65 years of age were selected from the Health Care Financing Administration file (Medicare). Live controls under age 65 were selected by telephone utilizing a two-staged random digit-dialing technique. For deceased cases the controls were selected from Kansas state mortality files with an additional match by year of death. Persons with a cause of death of STS, HD, NHL, homicide, suicide or malignancy of ill-defined site (ICD code 195) were excluded as controls.

Farm herbicide use was found to be non-significantly associated with NHL (OR = 1.6, 95% C.I. = 0.9, 2.6). The relative risk of NHL increased significantly with number of days of herbicide exposure per year and latency. Men exposed to herbicides more than 20 days per year had a sixfold increased

risk of NHL (OR = 6.0; 95% C.I. = 1.9, 19.5) relative to nonfarmers. Frequent users who mixed or applied the herbicides themselves had an OR of 8.0 (95% C.I. = 2.3, 27.9) for NHL. Dose response excesses were associated with use of phenoxyacetic acid herbicides, essentially synonymous with use of 2,4-D (OR = 2.3; 95% CI = 1.3, 4.3), since only three patients and 18 controls had used 2,4,5-T and all but two of these controls had also used 2,4-D. Use of 2,4-D only, i.e., eliminating 2,4,5-T users, was associated with an OR of 2.6 (95% CI = 1.4, 5.0). Neither STS nor HD was associated with herbicide or pesticide exposure. The authors concluded "this study confirms the reports from Sweden and several U.S. studies that NHL is associated with farm herbicide use, especially phenoxyacetic acids. It does not confirm the case-control studies or the cohort studies of pesticide manufacturers and Vietnam veterans linking herbicides to STS or HD". However, it is not contradictory to the hypothesis that 2,3,7,8-TCDD is the contaminant responsible for the development of STS, since 2,4-D does not contain 2,3,7,8-TCDD.

Few respondents could remember exposure to 2,4,5-T, which contains 2,3,7,8-TCDD. 2,4-D is not believed to contain 2,3,7,8-TCDD but does contain other polychlorinated dibenzo-p-dioxin impurities. The risk was found to increase with increasing frequency and duration of herbicide usage. Although "herbicide usage" could mean any of the herbicides identified by Hoar, she wrote that this is "essentially synonymous" with use of 2,4-D. The next most used herbicides i.e., triazines and uracils are nonsignificant when exposure to phenoxyacetic acids are controlled for in her analyses. However, this study has problems similar to the Hardell et al. (1981) study in that there is a lack of substantiation of exposure, and the information is based on questionnaire responses that are subject to some

recall bias. Moreover, there is a statistically significant risk associated with the use of other herbicides as well, i.e., triazines, amides, and trifluralin. These uncertainties, while raising concerns, do not discount the observed dose-response relationship or the observed increased incidence. A more detailed critical analysis of this study may be provided in a later version of this document.

Summary

Case reports of individuals who acutely ingested 2,4-D solutions indicate that early symptoms of exposure include gastritis, vomiting and loss of consciousness, and that muscular paralysis precedes death. Autopsies of fatal poisoning cases have shown widespread pathologic effects (e.g., congestion and hyperemia of most organs, hepatic necrosis). Reports of human poisoning from industrial or agricultural exposure to 2,4-D formulations (dermal and inhalation exposures) commonly described neurological signs and symptoms (e.g., fatigue, nausea, reflex disorders, paresthesia). Most of the clinical reports did not identify other possible chemicals to which the subjects may have been exposed, and did not indicate levels of 2,4-D exposure.

The epidemiologic studies of Hoar et al. (1986), Lynge (1985), Eriksson et al. (1981), Hardell et al. (1981), and Hardell and Sandstrom (1979) provide limited evidence of the carcinogencity of the phenoxy herbicides. The IARC (1987) has reviewed these studies and has also classified the phenoxy herbicides as having limited human evidence for carcinogenicity. The only study (Hoar et al., 1986) in which 2,4-D was specifically cited as the predominant herbicide, found a statistically significant association between exposure to 2,4-D and an elevated risk of non-Hodgkin's lymphoma.

This carefully designed and conducted study provides evidence of a dose-related excess risk of NHL with exposure to 2,4,-D. Because confidence in inferring a causal association from epidemiologic data is increased when several independent studies are concordant and with knowledge that NCI presently plans to release the results of two additional 2,4-D studies within 12 months, the CAG will withhold a weight-of-evidence judgement on the 2,4-D human data.

VII. MECHANISMS OF TOXICITY

The myotonic syndrome produced in rats by the administration of 2,4-D was studied by Ezyaguire et al. (1948). Intraperitoneal injection of 100-250 mg/kg of the 2,4-D sodium salt or intra-arterial administration of 2 mg of compound per animal produced neuromuscular effects that resembled those observed in congenital myotonia or those produced by poisoning with veratrine alkaloids. Muscles showed increased sensitivity to stimulating agents; a single spike was followed by a silent period and then a burst of repetitive firing. Increases in both twitch tension and twitch duration were noted. Constant activity of the affected muscles caused these myotonic symptoms to diminish, but following rest, the syndrome reappear.

Iyer et al. (1976) investigatec the neural factors contributing to this effect in a further study of the myotonic syndrome induced by intraperitoneal injection of 2,4-D in rats (dose unspecified). These investigators found that myotonic discharges appeared within 2 hours of a single injection and disappeared after 24 hours. Nerve block or nerve section produced after the initiation of myotonia had no effect on the condition; however, denervation of the muscle before treatment with 2,4-D produced a progressive loss of its myotonic response.

Iyer et al. (1977a,b, 1981) and Ranish et al. (1977) reported in later studies that rat skeletal muscle did not become myotonic following <u>in vivo</u> or <u>in vitro</u> exposure to 2,4-D if the muscle was previously denervated. They concluded that 2,4-D directly affects the muscle membrane (sarcolemma) and that the resting ionic conductance of sarcolemma is influenced by the presence of neural factors. Further evidence supporting this mechanism of

2,4-D toxicity was reported by Rudel and Senges (1972), following a study of intracellular recordings of membrane potential in rat diaphragm muscle exposed to 2,4-D; the sarcolemma was altered by decreased resting membrane conductance as a result of exposure to 2,4-D. Also, in a literature summary on myotonia in mammalian skeletal muscle, Kwiecinski (1981) noted the similarity between the conditions following the direct and selective activity of compounds such as 2,4-D on the sarcolemma of rats and those resulting from hereditary myotonia in humans. In humans, hereditary myotonia is generally thought to result from a genetic change in the structure and function of the sarcolemma.

Histochemical examination of skeletal muscle from rats in which myotonia had been induced by the intraperitoneal injection of 300 mg/kg 2,4-D indicated an inhibition of phosphorylase activity (Heene, 1966a). Further experiments by Heene (1966b) demonstrated that addition of $2x10^{-4}$ to $5x10^{-4}$ M concentrations of 2,4-D to 10 μm tissue sections of skeletal muscle would also inhibit phosphorylase and transglycosidase activities.

Dux et al. (1977) reported changes in the membrane of skeletal muscles from myotonic rats induced by intraperitoneal injection of 2,4-D (50 mg/kg/day for 21 days). Using a pyroantimonate precipitation technique to localize calcium in dystrophic muscle sections, these researchers were able to show lowered calcium in muscle triads and increased calcium associated with troponin C. They suggested that this shift in muscle calcium may lead to alterations in the actinomyosin contraction system. Seiler (1978) reported ~60% *in vitro* inhibition of both sodium-potassium and magnesium stimulated ATPases in sarcolemma extracts of normal rats following the

addition of 2.5 mM concentrations of 2,4-D. Direct injection of 100-500 mg/kg 2,4-D into the pectoralis muscle has been reported to increase muscle levels of glucose-6-phosphate within 30-45 minutes, but alteration in levels of ADPases and ATPases was not found (Kuhn and Stein, 1964). They further reported an inhibition of ^{45}Ca uptake by a sarcolemma preparation in vitro following the addition of 2,4-D.

Using embryonic chick fibroblasts prepared from primary muscle cultures, Emmons et al. (1980) investigated the relationship between perturbed sterol metabolism in the sarcolemma and experimentally induced myotonia. Application of 10 µg/mL of 2,4-D to the fibroblast cultures resulted in decreased cholesterol biosynthesis and an accompanying accumulation of acetate derivatives in desmosterol and related sterols. The authors concluded that the results obtained in this in vitro assay are consistent with the hypothesis that experimentally induced myotonia is preceded by a prerequisite change in steroid composition of the sarcolemma.

In vitro effects of 2,4-D on lipid biosynthesis in rat liver homogenates were reported by Olson et al. (1974). The addition of 4.5 and 9.0 mM concentrations of 2,4-D to liver preparations inhibited the incorporation of ^{14}C-mevalonate into nonsaponifiable lipids and, at the same concentrations, inhibited ^{14}C-acetate incorporation into cholesterol. The authors indicated that 2,4-D may produce hypolipidemic effects in a manner similar to that of chlorophenoxyisobutyrate, a clinically used agent. Kolberg et al. (1971) noted that L cell cultures exposed to 500 mg/mL 2,4-D for a 24-hour period showed increased incorporation of ^{3}H-palmitate and ^{14}C-acetate into total cell lipids. The increased ^{3}H-palmitate incorp-

oration was primarily in the triglyceride fraction, suggesting that increased fatty acid uptake from the medium was produced by 2,4-D at this level.

Brody (1952) reported that 2,4-D added *in vitro* to rat liver mitochondria was an uncoupling agent for oxidative phosphorylation. At concentrations as low as 5×10^{-5} M, 2,4-D began to decrease the phosphate/oxygen ratio in mitochondria without significantly affecting respiration, while at 1×10^{-3} M, more than 80% inhibition of phosphorylation was observed. Effects of 2,4-D on the oxidative phosphorylation of rat liver mitochondria were also studied by Whitehouse (1964), who reported no uncoupling effects at 2,4-D levels of 1×10^{-3} M; however, at 2.5×10^{-3} M, decreases in the phosphate/oxygen ratio were demonstrated. The differences in the inhibitory levels of 2,4-D reported by these two investigators are apparently due to the nature of the rat mitochondrial preparations tested, because Brody (1952) reports an almost 2-fold higher control value for phosphate/oxygen ratio than that reported by Whitehouse (1964). Weinbach and Garbus (1965) compared the uncoupling activity of several phenols in rat liver mitochondria and showed that complete uncoupling of oxidative phosphorylation in their assay system could be produced by 2×10^{-4} M dichlorophenol; dinitrophenol was effective in producing complete uncoupling at a 15-fold lower level. The 2,4-D uncoupling effects noted are therefore occurring at relatively high *in vitro* levels.

Cytotoxic effects of 2,4-D on cultured cells were observed by Haag et al. (1975). Exposure of cultured chick muscle cells to 2.5 mM 2,4-D for 44 hours produced moderate cytotoxicity that increased when the concentration

was raised to 5 mM. Morphological changes in cells treated at these 2,4-D levels included partial lack of polar orientation, diminished fiber formation and an increased nuclear/cytoplasmic ratio. Murakami and Fukami (1978) noted inhibition of cell growth in a human embryonic lung cell line when $4x10^{-6}$ M 2,4-D was present in cultures for 48 hours. Very little of the 2,4-D present in the medium was taken up by these cells (2.6-5.0 pmol/mg cell protein). Complete growth inhibition of L cells exposed for 24 hours to 350 mg/mL of 2,4-D has been reported by Kolberg et al. (1971).

Several investigations have demonstrated effects of 2,4-D on DNA synthesis, mitosis and cell-cycle parameters when added to cultured cells. Haag et al. (1975) observed that the S phase of the cell cycle was prolonged in cultured chicken muscle cells exposed to $2.5x10^{-3}$ M 2,4-D for 44 hours. Bongso and Basrur (1973) found that embryonic bovine kidney cells treated with 10 ppm of 2,4-D for 24 hours showed an elevated mitotic index caused by an increase in the number of prophase cells. Treated cells at 48 hours had increases in nucleolar size, nuclear lobulation, polyploid mitotic figures and multipolar spindles. The authors suggest that these changes may be produced by interaction of 2,4-D with mitotic spindle proteins. Increased mitotic activity has also been reported by Weiss and Beckert (1975) in monkey kidney cells and Girardi heart cells treated with 10 or 50 ppm of 2,4-D for up to 72 hours. Seiler (1979) found that oral administration of 2,4-D to mice can inhibit testicular DNA synthesis. One hour following intraperitoneal injection of ^{14}C-thymidine, mice were administered 200 mg/kg 2,4-D orally; 30 minutes later they were sacrificed and testicular DNA was extracted. Treated mice showed a 29% decrease in the incorporation of labeled thymidine into DNA. Inhibition of DNA synthesis *in vitro*, as

measured by the incorporation of ^{3}H-labeled deoxyhucleoside triphosphates into DNA by DNA polymerase, has been reported by Schwimmer (1968) when 1.5×10^{-4} M 2,4-D was added to the incubation mixture.

Enzyme inhibition by 2,4-D has also been reported in several other studies. Wedding and Black (1963) showed that porcine heart malic dehydrogenase, lactic dehydrogenase (rabbit muscle) and alcohol dehydrogenase (yeast) were all inhibited by 2,4-D at levels of $\sim 10^{-3}$ M. Inhibition was competitive relative to the pyridine nucleotide cofactors; the authors suggest that other pyridine nucleotide-requiring enzymes may be inhibited by 2,4-D. Increased activities of several placental enzymes have been observed in guinea pigs following maternal administration of 2,4-D (Humiczewska and Stanosz, 1971). Subcutaneous injection of 30 mg/kg of 2,4-D for 6 days/week throughout gestation increased succinate dehydrogenase, alkaline phosphatase and acid phosphatase activities as determined by histochemical methods.

Summary

Observations that rat skeletal muscle did not become myotonic following in vivo or in vitro exposure to 2,4-D if the muscle was previously denervated (Iyer et al., 1976, 1977a,b, 1981; Ranish et al., 1977) and intracellular recordings of membrane potential in rat muscle exposed to 2,4-D (Rudel and Senges, 1972) indicate that 2,4-D directly affects the sarcolemma. Changes in sarcolemma calcium (Dux et al., 1977; Kuhn and Stein, 1964), steroid (Emmons et al., 1980), and inhibition of muscle phosphorylase and transglycosidase activities (Heene, 1966a,b) were also observed in in vitro and in vivo experiments.

Addition of 2,4-D to rat liver preparations inhibited lipid biosynthesis (Kolberg et al., 1971; Olson et al., 1974) and oxidative phosphorylation (Brody, 1952; Whitehouse, 1964). Cytotoxicity (Kolberg et al., 1971; Haag et al., 1975; Murakami and Fukami, 1978), increased mitotic activity (Bongso and Basrur, 1973; Haag et al., 1975; Weiss and Beckert, 1975), and inhibition of DNA synthesis (Schwimmer, 1968; Seiler, 1979) and nucleotide-requiring enzymes (Wedding and Black, 1963) have also been observed in mammalian cells that were exposed to 2,4-D *in vitro* and *in vivo*.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \_\_\_\_ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner,

the U.S. EPA (1986) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{\text{(RfD) x (Body weight in kg)}}{\text{Drinking Water Volume in ℓ/day}} = \_\_\_\_ \text{ mg/ℓ}$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{\text{(NOAEL or LOAEL) x (bw)}}{\text{(UF) x (\_\_\_ ℓ/day)}} = \_\_\_\_ \text{ mg/ℓ}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a stucy of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these

estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is

uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

The different forms of 2,4-D (acids, salts, esters) have been discussed together in this document. Sufficient toxicokinetic data are not available to determine whether the esters should be considered separately from the acids and salts. There are no data to indicate how rapidly the esters of 2,4-D are hydrolyzed by mammals; however, at physiological pH, 2,4-D exists in the ionized form, which does not readily pass through biological membranes, as compared with the esters that would (Zetterberg, 1977). In humans, the rate of plasma uptake of orally administered 2,4-D, the degree of conjugation of the compound and the rate of elimination may vary considerably between individuals (Sauerhoff et al., 1977; Kohli et al., 1974); further characterization of this interindividual variation in the human population is needed.

The LD_{50} range of 2,4-D is generally between 380 and 1000 mg/kg; there does not appear to be significant differences in toxicity between the free acid and the various salt and ester derivatives. Hill and Carlisle (1947) determined oral LD_{50}s of 666, 375, 800 and 1000 for 2,4-D sodium salt in rats, mice, rabbits and guinea pigs, respectively; the maximum doses in these species not causing death were 333, 125, 200 and 333 mg/kg, respectively. Individual monkeys that were fed single doses of ~286 or 428 mg/kg of 2,4-D sodium salt or 286 mg/kg of 2,4-D ammonium salt regurgitated a large portion of the material, precluding determinations of lethal doses (Hill and Carlisle, 1947). Symptoms other than nausea were not described in these monkeys. Approximately 214 mg/kg of 2,4-D sodium salt was fed to another monkey without development of vomiting or "serious illness" (Hill and Carlisle, 1947). Comparison of the species sensitivity to 2,4-D indicates that dogs may show greater sensitivity to this compound (Rowe and Hymas, 1954). The higher toxicity observed in dogs may reflect an inability of kidney processes in this species to effectively clear phenoxyacetic acids (Seiler, 1978).

Drill and Hiratzka (1953) described myotonia with pathologic changes of GI mucosa irritation, moderate hepatic necrosis and mild renal tubular degeneration in dogs that were lethally poisoned by acute oral administration of 2,4-D at doses of 100-400 mg/kg.

Bucher (1946) found that myotonia persisted for 8-24 hours in strain A mice that were injected intraperitoneally with sublethal doses (100-200 mg/kg of 2,4-D). No significant differences were found in the effects produced when 2,4-D was administered subcutaneously, intraperitoneally or intravenously.

In a summary report, Rowe and Hymas (1954) described an experiment where doses of 0, 30, 100 or 300 mg/kg/day 2,4-D to groups of 5 or 6 female rats (strain not specified) by intubation 5 times/week for 4 weeks were utilized. The 2,4-D was administered in olive oil that was emulsified in 5-20% aqueous gum arabic, and the controls were vehicle treated. Rats that received 30 mg/kg/day or less reportedly showed no adverse treatment-related clinical or pathological effects, but treatment with 100 mg/kg/day elicited GI irritation, depressed growth rate and slight cloudy swelling of the liver. Rats that received 100 mg/kg/day 2,4-D succumbed rapidly (not elaborated) and died; severe GI irritation was reportedly the principal adverse effect observed.

In addition, groups of five young adult female rats (strain not specified) were maintained on diets that contained 0, 100, 300 or 1000 ppm 2,4-D in the diet for 113 days (Rowe and Hymas, 1954). If it is assumed that young rats consume 10% of their weight in food per day, the corresponding daily doses are 0, 10, 30 and 50 mg/kg/day. Rats that were exposed at the 1000 ppm level experienced excessive mortality (not quantified), depressed growth rate, excessive mortality and slight cloudy swelling of the liver. These effects were not observed at the two lowest doses. Groups of five rats that were given diets that contained higher concentrations of 2,4-D (3000 or 5000 ppm) were sacrificed after 12 days because they were not eating and were rapidly losing weight; examinations revealed increased liver and kidney weights and slight but unspecified pathologic changes.

Chang et al. (1974) reported that dietary administration of 2,4-D to rats at levels of 2000 ppm in the diet for 4-7 (~200 mg/kg/day) weeks

produced a slight increase in liver glycogen content, a slight decrease in liver RNA content and slight decreases in absolute and relative liver weights, but no overt signs of toxicity.

Administration of 0, 200 or 400 ppm dietary 2,4-D (~0, 10 or 20 mg/kg/day, respectively) to groups of seven rats for 1 month did not adversely affect food intake or rate of growth, or elicit characteristic signs of intoxication (skeletal muscular signs or paralysis) (Hill and Carlisle, 1947). Dietary administration of 2,4-D at a level of 100 ppm for 21 days and subsequently 1000 ppm for 10 days (average total dose ~39.0 mg/kg/day) was similarly non-toxic for rats. Groups of six guinea pigs that were given 10 daily doses of 50 or 100 mg 2,4-D in 12 days (~88 or 177 mg/kg/day) by intubation also did not develop characteristic evidence of intoxication.

Drill and Hiratzka (1953) administered 2,4-D orally in capsules to groups of 2-4 dogs at doses of 0, 2, 5, 10 or 20 mg/kg/day, 5 days/week, for 13 weeks. When adjusted for a 7-day week, the respective daily doses are 0, 1.4, 3.6, 7.1 and 14.3 mg/kg/day. Toxic effects were only observed at the high dose. Treatment at 20 mg/kg/day produced death in 3 or 4 dogs between days 18 and 49, and symptoms in the moribund animals included hind leg stiffness, ataxia, weakness, gum bleeding and difficulty in chewing and swallowing. A terminal decrease in the percentage of blood lymphocytes was noted in the 3 dogs that died, but significant effects on the hemoglobin, red cell count or total white cell count were not observed. The dog that survived 2,4-D treatment at the high dose, as well as the dogs exposed to the lower levels of 2,4-D, showed no significant hematologic, gross or histopathologic effects.

Effects of subcutaneously injected 2,4-D (sodium salt) on the thyroid gland of treated rats have been reported (Florsheim and Velcoff, 1962; Florsheim et al., 1963). These investigators showed that, following seven daily injections of 2,4-D at a level of 100 mg/kg, thyroid weight was decreased. Administration of 2,4-D at 80 mg/kg over this period increased radioactive iodine uptake by the thyroid, lowered the binding of radiolabeled thyroxine by serum proteins, and increased the amount of radiolabeled compound in the liver of treated rats.

Toxic effects of 2,4-D on the nervous system of rats administered lethal doses of the compound intraperitoneally have been described by Desi et al. (1962). Animals injected daily with 200 mg/kg of 2,4-D (form not specified) died within 6 days. Progressively decreased conditioned reflex responses were observed over this period, as well as the appearance of large slow waves in the EEG. Histological examination indicated that demyelinization was present in the dorsal portion of the spinal tract. Within 10-15 minutes following a single intraperitoneal injection of the compound, EEG changes were observed (decreased cerebral and reticular desynchronization); recovery was seen in ~1 hour. The authors postulate that the neurological effects produced by 2,4-D in this study are due initially to action of the compound on the reticular formation, followed by later effects on cerebral tissue. Histological examination, however, failed to show any morphological changes in the cortex or subcortical regions of treated animals. The demyelinization observed in the spinal cord may be responsible for the hind limb paralysis noted by other investigators after poisoning of animals with 2,4-D.

Hansen et al. (1971) conducted 2-year feeding studies with technical grade (96.7% pure) 2,4-D in Osborne-Mendel rats. In the rat study, 25 animals of each sex were exposed to 0, 5, 25, 125, 625 or 1250 ppm 2,4-D in the diet (~0, 0.25, 1.25, 6.25, 31.25 or 62.5 mg/kg/day) from 3 weeks of age. At the conclusion of treatment, all rats were autopsied, but comprehensive histopathologic examinations were performed only on 6 rats/sex from the high-dose and control groups; the liver, kidneys, spleen ovaries or testes and other tissues that contained gross lesions were histologically examined in the remaining rats in the high exposure and control groups and in the rats at the other dose levels. Significant differences in survival, mean bw and organ/bw ratios (liver, kidney, heart, spleen or testes) were not found between any of the treated groups and the control group during the 2-year treatment or at the end of the study. Significant treatment-related pathologic effects were not observed. The incidence of tumors did not differ significantly between the groups. Several hematologic indices (hemoglobin, hematocrit, total white cell count) were similar in the treated and control groups, but it was noted that the red blood cell count of the treated rats (1250, 625 and 5 ppm groups) showed a "tendency" toward macrocytosis, "very slight to slight" polychromasis, and "slight to moderate" hypochromasia. The tendency toward macrocytosis was reportedly not present and the other red cell abnormalities were of a "minor degree" in the control rats. The toxicological significance of these vaguely reported effects is unclear.

In a two-generation reproduction study with rats that is also discussed in Other Reproductive Effects in Chapter V (Bjorklund and Erne, 1966), administration of 1000 ppm 2,4-D in the drinking water (~50-100 mg/kg/day)

of rats (5/group) during pregnancy and for a further 10 months had no significant effects on the maternal animals (not specified) or offspring (clinical signs or malformations). Similar exposure of 22 weaned offspring (10 males, 12 females) for up to 2 years was, with the exception of reduced food and water intake and consequent growth retardation, temporary diarrhea and poor general condition, also nontoxic as judged by normal clinical chemistry indices, hematocrit, hemoglobin, plasma GOT, plasma elimination rate of 2,4-D (3 hours), relative organ weights (heart, spleen, liver, kidneys, lungs, testes, ovaries), or gross or microscopic pathology. Other reproduction studies that are detailed in Teratogenicity and Other Reproductive Effects in Chapter V reported that dietary exposure to 1500 ppm (~75 mg/kg bw) 2,4-D for 2 years (Hansen et al., 1971) and dietary exposure to 1000 ppm (~100 mg/kg) for 3 months (Gaines and Kimbrough, 1970) prior to mating and during pregnancy and lactation caused an increase in preweanling mortality.

Hansen et al. (1971) also fed 6- to 8-month-old beagle dogs (3 of each sex/group) 0, 10, 50, 100 or 500 ppm technical grade 2,4-D in the diet (~0, 0.29, 1.45, 2.9 or 14.5 mg/kg/day) for 2 years. Treatment-related effects were not indicated based on observations of mortality as well as gross and microscopic tissue examinations in any of the treated groups or control group.

Quantification of Noncarcinogenic Effects

Derivation of 1-Day HA. Clinical reports have described cases of human ingestion of 2,4-D and its derivatives (see Acute Effects in Chapter VI), but there is only one report of nonfatal poisoning in which quantitative exposure data were provided. Berwick (1970) described a farmer who

swallowed a mouthful of weed killer (49% Eptam, 35.5% 2,4-D isooctyl ester, 0.5% epichlorohydrin and 5% emulsifiers) and exhibited characteristic symptoms of 2,4-D exposure (e.g., acute gastritis, vomiting, skeletal muscle damage) that subsided within ~2 weeks. There was no evidence of cholinesterase inhibition, further indicating that the toxicological effects were not induced by the Eptam. If it is conservatively assumed that the volume of a mouthful of liquid is 25 mℓ and that the density of 2,4-D isooctyl ester is similar to that of 2,4-D n-butyl ester (1.2 g/mℓ), the quantity of isooctyl ester ingested can be estimated to be ~11 g (25 mℓ x 0.355 x 1.2 g/mℓ); the equivalent quantity of 2,4-D acid would be ~6.7 g (~96.7 mg/kg, assuming a body weight of 70 kg). Although factors such as the crude quantitation of the quantity ingested and the unknown pharmacokinetic properties of 2,4-D isooctyl ester could preclude the identification of a human single-dose nonlethal level from this study, a more serious problem is a lack of corroborating data. In other case reports, deaths were described in a male agricultural student who ingested at least 6 g of a commercial herbicide preparation of (50% w/w) 2,4-D dimethylamine salt ($\geq$2.5 g 2,4-D acid equivalent) (Nielson et al., 1965) and in a 46-year-old man who died within 14 hours of swallowing at least 13.5 g of an uncharacterized 2,4-D solution (Herbich and Machata, 1963). It is apparent from examination of these data that the exposures in the above reports are too poorly characterized to be used to identify an unequivocal nonlethal dose that could be used to derive a 1-day HA; the estimated nonlethal adverse effect level in the Berwick (1970) report (~6.7 g) appears to be bracketed by the apparent lethal doses of Nielson et al. (1965) ($\geq$2.5 g) and Herbich and Machata (1963) ($\geq$13.5 g).

Most of the information on the effects of single oral exposures to 2,4-D and derivatives in laboratory animals is related to lethality (see Table VI-1). Hill and Carlisle (1947), however, reported the results of toxicological studies in which both lethal doses and tolerated doses (the largest dose that caused no deaths) for 2,4-D sodium salt were determined for four species of rodents. The lethal and tolerated oral doses for 2,4-D sodium salt in rats, mice, guinea pigs and rabbits were 666 and 333 mg/kg bw, 375 and 125 mg/kg bw, 1000 and 333 mg/kg bw, and 800 and 200 mg/kg bw, respectively. These data indicate that mice are the most sensitive species, but it must be noted that a tolerated dose, as defined in this study, does not imply a NOAEL. Although the tolerated doses caused no deaths, effects other than survival were not mentioned; symptoms and pathological changes were only specifically described in the animals that died (i.e., the lethal dose groups). Since Hill and Carlisle (1947) noted that small ranges of doses were tested (i.e., groups of 10 mice were administered 125, 250, 375 and 500 mg/kg bw of 2,4-D sodium salt), it appears probable that some of the characteristic signs or symptoms of intoxication described at the lethal doses (e.g., muscular effects and possible histological damage) would also have been evident in those animals given tolerated doses. A 1-day HA can be calculated from the tolerated single dose for mice (125 mg 2,4-D sodium salt/kg bw, equivalent to about 114 mg 2,4-D/kg bw) using an uncertainty factor (UF) of 1000. This factor, as per previous guidelines (U.S. EPA, 1980), represents 10-fold for both intra- and interspecies variability to the toxicity of a chemical when specific data are lacking and an additional 10-fold because the tolerated single dose is assumed to have caused unreported adverse effects and, therefore, is considered a LOAEL rather than a NOAEL.

Thus, for a child:

$$\text{1-day HA (child)} = \frac{(114 \text{ mg/kg bw/day} \times 10 \text{ kg bw}) \div (1000 \times 1 \text{ ℓ/day})}{\sim 1.1 \text{ mg/ℓ}}$$

This HA is equivalent to 1.1 mg/day or 0.1 mg/kg bw/day.

A 1-day HA can alternatively be derived from other data of Hill and Carlisle (1947). In this experiment, groups of six guinea pigs that were administered 10 doses of 50 or 100 mg/day 2,4-D sodium salt by gavage in 12 days did not develop characteristic evidence of intoxication (i.e., muscular signs) or mortality. If it is assumed that the guinea pigs weighed 0.3 kg (the reported approximate weight in the single dose studies), the lowest reported no effect dose of 50 mg/day corresponds to a daily dose of about 139 mg/kg bw/day (50 mg/day ÷ 0.3 kg bw x 10/12); the equivalent dose of 2,4-D acid is about 126 mg/kg bw/day. Although symptoms or signs of intoxication were not specifically associated with this exposure, these criteria of toxicity are still too insensitive to justify using 126 mg/kg bw/day as an animal NOAEL with an uncertainty factor of 100. If 126 mg/kg bw/day is regarded as a LOAEL (reasoning similar to representing the single tolerated doses as LOAELs) and an uncertainty factor of 1000 is used, the 1-day HA value for a child is approximately the same as the 1-day HA derived from the single tolerated dose for mice:

$$\text{1-day HA (child)} = \frac{(126 \text{ mg/kg bw/day} \times 10 \text{ kg bw}) \div (1000 \times 1 \text{ ℓ/day})}{\sim 1.3 \text{ mg/ℓ}}$$

This HA is equivalent to 1.3 mg/day or 0.13 mg/kg bw/day.

Either one of the above cited experiments can be used to estimate the 1-day HA. They are both of equal value and the calculated concentrations are essentially the same.

Derivation of 10-Day HA. Subchronic or shorter duration studies may be used to calculate a 10-day health advisory. The Health Effects Branch of the Office of Drinking Water has assessed the available data with the objective of estimating a 10-day health advisory and discovered several deficiencies. The National Academy of Sciences (NAS, 1977) also concluded, "There are substantial disagreements in the results of subchronic and chronic toxicity studies with 2,4-D, perhaps reflecting the use of different formulations or preparations". This section provides critical evaluation of data and attempts to generate a 10-day health advisory.

Limited human information is available that provides quantitative short-term exposure data. Kephart (1945) moderated a discussion in which an individual reported that he had taken (presumably orally) 500 mg of 2,4-D/day for 21 days with no demonstrable ill-effects. Additional information was not available, but this exposure corresponds to a daily dose of ~7 mg/kg bw if it is assumed that the person weighed 70 kg. Seabury (1963) reported a case in which a patient was administered 18 intravenous doses of 2,4-D (with indole butyric acid and naphthalene acetic acid) over a 33-day period for the treatment of coccidiodomycosis. Infusion of a total of 10.7 g did not produce observed side effects (the dosage was 800 mg/day for doses 9 through 17, and the 18th dose was increased to 2000 mg). A final 19th dose of 3.6 g that was infused over 2 hours (~67 mg/kg) elicited fibrillary muscle twitching and general hyporeflexia that completely subsided within 48 hours. Although these reports may provide a crude indication of a human tolerated dose, numerous factors preclude their use in deriving a 10-day HA [e.g., anecdotal nature of the inadequately reported Kephart (1945) data, the inappropriate route of administration in the Seabury (1963) study, the lack of sensitive indicators of toxicity].

Rowe and Hymas (1954) administered 2,4-D (purity unspecified in olive oil/gum arabic vehicle) by gavage to groups of 5 or 6 young adult female rats at doses of 0, 3, 30, 100 or 300 mg/kg bw for 5 days/week for 4 weeks (0, 2.14, 21.4, 71.4 and 214 mg/kg bw/day, respectively). Adverse effects as judged by gross appearance and behavior, growth, hematological values (not elaborated), blood urea-nitrogen concentrations, organ weights, gross and histopathological examinations (tissues not reported) and mortality were not observed at doses of 2.14 or 21.4 mg/kg bw/day. Gastrointestinal irritation, slight cloudy swelling of the liver and depressed growth rate were apparent at 71.4 mg/kg bw/day, and rats that were administered 214 mg/kg bw/day 2,4-D died; the time to death was not reported, but severe GI irritation was the principal effect observed (other pathological effects were not discussed). In another study of longer duration, the same investigators (Rowe and Hymas, 1954) administered 0, 100, 300 or 1000 ppm 2,4-D in the diet to groups of five young female rats for 114 days. If it is assumed that young rats consume 10% of their body weight in food per day, the corresponding daily doses would be 0, 10, 30 and 100 mg/kg bw/day. No effects (same indices as in the 4-week gavage study) were found at 10 or 30 mg/kg bw/day, but 100 mg/kg bw/day produced "excessive mortality" with depressed growth rate, slightly increased liver weights and slight cloudy swelling of the liver. Rats exposed to higher levels of 2,4-D in the diet (3000 and 5000 ppm) were not evaluated because they refused food and consequently lost weight and the experiment was terminated. Both of the above Dow Chemical Company studies used small groups of animals and were not reported in detail, but multiple dose levels were tested and a number of toxicity indices were evaluated.

In another briefly reported dietary study with rats (Hill and Carlisle, 1947), adverse effects as judged by signs of intoxication, decreased food consumption, growth impairment or mortality were not observed in groups of seven rats that were exposed to purified commercial 2,4-D at daily doses as high as ~40 mg/kg bw/day (200 or 400 ppm in the diet for 31 days, 100 ppm in the diet for 21 days and 1000 ppm in the diet for the subsequent 10 days). Although this study did not examine sensitive toxicity indices (e.g., clinical chemistry or hematology indices, histopathology), the reported no effect level of ~40 mg/kg bw/day is consistent with that reported by Rowe and Hymas (1954) after 4 weeks of gavage exposure (21.4 mg/kg bw/day) or 16 weeks of dietary exposure (30 mg/kg bw/day). Chang et al. (1974) found in a single dose study with an unspecified number of rats (~8) that dietary exposure to 2000 ppm analytical grade 2,4-D (~200 mg/kg bw/day) for 4-7 weeks produced mild liver effects (slight increase in glycogen, slight decrease in RNA, slight decrease in absolute and relative weights) but no effect on food consumption or overt signs of toxicity. The results of this study are somewhat supportive of the 71.4 mg/kg bw/day LOAEL in the Rowe and Hymas (1954) 4-week gavage study (cloudy swelling in liver, GI irritation, depressed growth) but are not consistent with the Rowe and Hymas (1954) 16-week diet study that found excessive mortality at 100 mg/kg bw/day and food refusal at higher levels.

In another short-term oral study with a limited number of animals (Drill and Hiratzka, 1953), dogs were given commercial grade 2,4-D in capsules at doses of 0 (2 females), 2 (1 male, 1 female), 5 (1 male, 1 female), 10 (3 males) or 20 mg/kg bw (3 males, 1 female) 5 days/week for 13 weeks (0, 1.4, 3.6, 7.1 or 14.3 mg/kg bw/day, respectively). The dogs survived exposure to

≤7.1 mg/kg bw/day without symptoms or changes in body weight, organ weights, hemoglobin content, blood count, or gross or microscopic tissue structure, but 3 of the 4 dogs that received 14.3 mg/kg/day died with neuromuscular symptoms and terminal lymphocytosis (but no significant pathological lesions). The FEL in this study (14.3 mg/kg bw/day) is therefore lower than the highest NOAELs reported in the short-term rat studies (Rowe and Hymas, 1954; Hill and Carlisle, 1947). Although corroborating short-term exposure data in dogs are not available, both acute oral lethal (Drill and Hiratzka, 1953) and longer-term oral (Hansen et al., 1971) studies also indicate that dogs are more sensitive than rats; however, 14.5 mg/kg bw/day was reported by Hansen et al. (1971) to be a 2-year oral NOAEL for dogs (see Assessment of Chronic Oral Data and Derivation of a Lifetime Adjusted Acceptable Daily Intake in this chapter).

Teratogenicity testing with rats, mice and hamsters has shown that oral administration of 2,4-D during gestation may produce fetotoxic and developmental effects at daily doses that are in the range of the rat and dog subchronic NOAELs. Khera and McKinley (1972) found increased fetal mortality and an increased incidence of skeletal malformations in rats following oral administration of 2,4-D and 2,4-D esters and salts at 100 mg/kg bw/day (or higher levels) on days 6 through 15 of gestation. Fetal mortality was not elevated at lower dosages but the incidence of skeletal malformations was slightly elevated by 2,4-D at 25 or 50 mg/kg bw/day. This increase was significant ($p<0.05$) at the 25 but not at the 50 mg/kg/day level. Schwetz et al. (1971) reported similar types of effects in rats after administration of 2,4-D or its PGBE or isooctyl ester at 75 or 87.5 mg 2,4-D/kg on days 6 through 15 of gestation. Lower doses of 12.5 or 25 mg/kg on days 6 through

15 of gestation produced statistically significant increases in the incidences of some developmental effects (e.g., delayed ossification, missing sternebrae, and subcutaneous edema), although the incidences of these effects among different control groups were variable. For 2,4-D specifically, a significant increase in the incidence of delayed ossification of skull bones was reported at doses of 12.5, 50, 75 and 87.5 mg/kg bw/day but not at 25 mg/kg bw/day. The author reported that the incidence of this response at 12.5 mg/kg bw/day was lower than the spontaneous incidence in the second control group of this study. Furthermore, at these levels of treatment, generalized fetotoxic effects were not seen. Jnger et al. (1981), in a similar study with rats, detected slight fetotoxicity at 87.5 mg 2,4-D/kg, administered as the PGBE or isooctyl ester, but no effects were detected at lower doses (<25 mg/kg). Courtney (1977) observed an increased incidence of cleft palate formation and increased fetotoxicity in CD-1 mice after oral administration of 221 mg/kg 2,4-D or equimolar levels of the PGBE or n-butyl esters on days 12 through 15 of gestation. The most toxic 2,4-D ester in this assay, PGBE ester, produced cleft palates and fetotoxic effects at a dose equivalent to 124 mg 2,4-D/kg/day, while this same lower level of the isopropyl or isooctyl esters produced fetotoxic effects but no increase in cleft palates. Thus, at 2,4-D levels that are in the approximate range of half the LD_{50} value for mice, some incidences of cleft palates have been observed. Collins and Williams (1971) were unable to show teratogenic effects in hamsters following oral administration of commercial 2,4-D preparations on days 6 through 10 of gestation at levels up to 100 mg/kg; fetotoxic effects were observed at 40, 60 and 100 mg/kg/day, but not at 20 mg/kg/day.

These teratogenicity tests for 2,4-D and its esters indicate that oral doses (expressed as 2,4-D) of 40, 60 or 100 mg/kg bw/day in hamsters on days 6 through 10 of gestation (Collins and Williams, 1971), of 75-125.5 mg/kg bw/day in rats on days 6 through 15 of gestation (Schwetz et al., 1971; Unger et al., 1981; Khera and McKinley, 1972) and of 124 mg/kg bw/day in mice on days 7 through 15 of gestation (Courtney, 1977) produced fetotoxic effects or malformations. The threshold for adverse effects on the fetus is not clearly defined: sporadic evidence of mild fetotoxicity was reported in rats at doses as low as 12.5 and 25 mg 2,4-D/kg bw/day (Schwetz et al., 1971; Khera and McKinley, 1972) for both 2,4-D and 2,4-D esters, but these effects were also seen in controls. Furthermore, generalized fetotoxic effects were not seen at these levels of treatment.

Because these teratogenicity studies have shown evidence of adverse fetal effects at daily doses that are higher than the subchronic NOAELs, one of these latter NOAELs would be the most appropriate basis for derivation of a 10-day HA. The NOAEL chosen here is 30 mg/kg bw/day (Rowe and Hymas, 1954). This NOAEL is the highest available based on several toxicity endpoints including histopathological analysis. The lower FEL of 14.3 mg/kg bw/day in a limited number of dogs (Drill and Hiratzka, 1953) is not considered relevant to this analysis, because it is contradicted by a 2-year feeding study with a large number of dogs (Hansen et al., 1971).

Using the same assumptions as in the 1-day HA calculation, a 100-fold uncertainty factor for an animal NOAEL, and an additional 10-fold safety factor for deficiencies in the chosen study, a 10-day HA is derived for a child as follows:

$$\text{10-day HA (child)} = \frac{(30 \text{ mg/kg bw/day} \times 10 \text{ kg bw}) \div (100 \times 10 \times 1 \text{ ℓ/day})}{\sim 0.30 \text{ mg/ℓ}}$$

This HA is equivalent to 0.30 mg/day or 0.030 mg/kg bw/day.

Derivation of Longer-term HA. A longer-term HA has not been calculated because of the lack of appropriate data.

Assessment of Lifetime Exposure and Derivation of a DWEL. Lifetime DWELs are normally derived from 2-year feeding studies in animals. The animal species that is most sensitive to the toxic effects or the species that metabolizes the compound in a manner similar to that in man is selected for estimating DWELs for humans. In these studies, a no adverse health effects level is identified. This level is divided by an uncertainty factor which may vary from 10-1000 based on the overall scientific judgment to determine an DWEL.

In 1976, the U.S. EPA, Office of Drinking Water, established an interim primary drinking water standard of 0.1 mg 2,4-D/ℓ. This was developed from an article -- Summaries of Pesticide Toxicity by Lehman. A lowest long-term level of 8 mg/kg bw/day with minimal or no effects in dogs was identified. A safety factor of 500, an average daily intake of 2 ℓ of water by man and 20% of the total acceptable daily intake were the other factors taken into consideration to derive the final standard.

The National Academy of Sciences (NAS, 1977) recommended a concentration of 0.09 mg 2,4-D/ℓ in drinking water, assigning 20% of the total RfD to the drinking water source. This recommendation was based on a study in dogs by Hansen et al. (1971). The Academy applied an uncertainty factor of 1000 to the NOAEL in dogs, recognizing the deficiencies in the study. The Academy's calculations are given below:

$$\frac{12.5 \text{ mg/kg} \times 70 \text{ kg} \times 0.2}{2 \text{ ℓ} \times 1000} = 0.09 \text{ mg/ℓ}$$

where: 12.5 mg/kg = no adverse effect level
70 kg = assumed average body weight of an adult
0.2 = factor representing 20% of total intake from water
2 ℓ = assumed average daily intake of water for man
1000 = uncertainty factor due to inter- and intraspecies variations and deficiencies in the studies

Johnson (1971) indicated that 220 Dow Chemical Company production workers who were exposed to 2,4-D in the range of 30-40 mg/day (~0.4-0.6 mg/kg bw/day, assuming a weight of 70 kg) over a period of 0.5-22 years showed no significant clinical effects when compared with an unexposed population. These data are from an unpublished study and are inadequate as reported for derivation of a lifetime DWEL. Particularly, additional information is needed regarding the exposure estimate (which presumably reflects inhalation and dermal exposures) and the effects (it was reported only that a battery of "at least 10 laboratory tests" was conducted). Singer et al. (1982) reported that nerve conduction velocities were slowed in workers who were engaged in the manufacture of 2,4-D and 2,4,5-T for an average of 7 years, but exposure levels were not determined.

Hansen et al. (1971) fed technical grade 2,4-D to groups of 25 Osborne-Mendel rats of each sex at levels of 0, 5, 125, 625 and 1250 ppm (0, ~0.25, 6.25, 31.25 and 62.5 mg/kg bw/day, respectively, assuming corsumption of 5% of body weight in food/day) for 2 years. Adverse effects on growth, survival, organ weights, tissue histology or hematological values were not attributed to exposure at any of the treatment levels up to and including ~62.5 mg/kg bw/day. Although all rats were autopsied at the end of the 2-year test, comprehensive histological examinations were performed only on six rats of each sex from the high-dose and control groups; histological examinations in the remaining rats from the high-dose and control groups and in the rats in the other groups were limited to the liver, kidney, spleen, ovary or testis and other tissues that contained gross lesions.

In a similarly designed study with dogs, 6- to 8-month-old beagles (3 of each sex/group) were fed either 0, 10, 50, 100 or 500 ppm in the diet for 2 years (Hansen et al., 1971). If it is assumed that dogs consume 2.9% of their weight in food/day, the corresponding daily doses would be 0, 0.29, 1.49, 2.9 and 14.5 mg/kg bw/day, respectively. Treatment-related gross or histopathological effects were not associated with any of the exposures. Twenty-eight of the 30 treated dogs survived the test period and were clinically normal, but the report did not state if hematological analyses were performed as in the rat study.

A 3-generation, 6-litter reproduction study was also conducted by Hansen et al. (1971) in Osborne-Mendel rats. A decrease in average preweanling weight and in the survival of offspring during the first 3 weeks after birth was observed when test animals were maintained on 1500 ppm 2,4-D in the diet

(~75 mg/kg bw/day) for 2 years. These effects were not observed at 500 ppm (~25 mg/kg bw/day) or 100 ppm (~5 mg/kg bw/day). These authors also reported an unpublished study by Gaines and Kimbrough (1970) in which feeding 1000 or 2000 ppm 2,4-D in the diet (~50 or 100 mg/kg bw/day, respectively) to rats for 3 months before and during pregnancy and lactation resulted in increased mortality among the offspring. Bjorklund and Erne (1966), however, found no significant effects on dams or their offspring following administration of 1000 ppm 2,4-D in drinking water (50-100 mg/kg bw/day) to pregnant rats throughout gestation and for 10 months beyond parturition, and to the offspring for up to 2 years.

The chronic toxicity and reproduction studies of 2,4-D indicate no adverse effects at dietary levels up to 500 ppm in dogs (~14.5 mg/kg bw/day), up to 1250 ppm in rats (~62.5 mg/kg bw/day) (Hansen et al., 1971) or at levels of 1000 ppm in drinking water (50-100 mg/kg bw/day) in pregnant rats (exposed throughout gestation and for 10 months following parturition) or their offspring (exposed for up to 2 years after weaning) (Bjorklund and Erne, 1966). As previously discussed, however, a secondary reference to another study reported an increase in mortality among young rats whose dams received ~50 mg/kg bw/day of 2,4-D in the diet for 3 months before mating and throughout gestation and lactation (Gaines and Kimbrough, 1970). Moreover, the subchronic study of Hazelton Laboratories (1983) indicates that these chronic studies may not be the most appropriate basis for the derivation of an RfD. Hazelton Laboratories (1983) report multiple adverse effect at subchronic doses of 5.0 or 15 mg/kg bw/day, or higher in both mice and rats. A dose of 1.0 mg/kg bw/day was reported as a NOAEL in rats. In addition, preliminary results from a 2-year bioassay indicate no change in this latter NOAEL.

Giving consideration to all of these studies, it seems reasonable to estimate a lifetime DWEL for a 70 kg human from the rat NOAEL of 1.0 mg/kg bw/day of Hazelton Laboratories (1983) using an uncertainty factor of 100. This factor represents a 10-fold decrease in dose for both intra- and inter-species variability to the toxicity of a chemical when specific data are lacking.

$$\text{Lifetime DWEL} = (1.0 \text{ mg/kg bw/day} \times 70 \text{ kg bw}) \div (100 \times 2 \text{ L/day}) = 0.35 \text{ mg/L}$$

This HA is equivalent to an RfD of 0.70 mg/day or 0.010 mg/kg bw/day.

There are discrepancies in the results of the subchronic and chronic toxicity studies. The highest NOAEL for rats in the 2-year Hansen et al. (1971) study (62.5 mg/kg bw/day), for example, is higher than the highest NOAELs reported in the subchronic rat studies of 30 mg/kg bw/day of Rowe and Hymas (1954) and of 40 mg/kg bw/day of Hill and Carlisle (1947), although this result may only be due to the doses employed. The Hansen et al. (1971) rat NOAEL is also close to the frank-effect level (100 mg/kg bw/day) in the 113-day experiment (Rowe and Hymas, 1954). The highest NOAEL for dogs in the Hansen et al. (1971) 2-year study (14.5 mg/kg bw/day) is similar to the FEL (14.3 mg/kg bw/day) in the 13-week dog study (Drill and Hiratzka, 1953). Furthermore, recent data by Hazelton Laboratories (1983) indicate that subchronic doses as low as 5 mg/kg bw/day might represent adverse effect levels in rats and perhaps mice. These variable results may reflect the use of different 2,4-D formulations or the inadequacies of the short-term studies as previously discussed (e.g., small numbers of animals per dose, inadequate reporting of data). These differences in 2,4-D toxicity could also possibly be due to a sharp break in the no-effect, effect dose region. These differences may be resolved by additional testing.

Conclusions. A summary of the data used to calculate the HAs and the lifetime DWEL is provided in Table VIII-1. The values derived for the HAs and the DWEL represent estimates of the concentrations of 2,4-D in drinking water that will not cause adverse effects after 1 day, 10 days or lifetime exposures.

Carcinogenic Effects

Quantification of Carcinogenic Effects

The available animal and epidemiology carcinogenicity studies have not conclusively shown that 2,4-D alone is carcinogenic. Administration of 2,4-D or the butyl, isopropyl or isooctyl esters of 2,4-D by intubation (46-100 mg/kg bw/day) on days 7 through 28 of age and subsequently in the diet (111-323 ppm, ~14-42 mg/kg bw/day) for up to 90 weeks was not tumorigenic for mice (Bionetics Research Lab., 1968b). Administration of 2,4-D in the diet at levels as high as 1250 ppm (~62.5 mg/kg/day) for 2 years was originally reportedly not carcinogenic for rats (Hansen et al., 1971), but later examination of the histology sections by Reuber (1979) found a significant increase in the incidence of lymphosarcomas in females at all dose levels; histopathologic reevaluation and consideration of the spontaneous incidence of lymphoid tumors in Osborne-Mendel rats are needed to resolve the discrepancy. A Russian study reported that administration of 2,4-D amine salt in the diet for life at one-tenth the LD_{50} (level not specified) was not tumorigenic for rats or mice (Archipov and Kozlova, 1974). Single subcutaneous injections of 2,4-D (215 or 464 mg/kg bw), 2,4-D isopropyl ester (100 mg/kg bw) or 2,4-D isobutyl ester (21.5 mg/kg bw) were not tumorigenic for mice after 78 weeks, but similar injection of 2,4-D isooctyl ester (21.5 mg/kg bw) induced a significant increase in reticulum

TABLE VIII-1

Summary of Data Used to Derive HA and DWEL

Criteria	Animal Dose	Duration	Effect	Value of HA or AADI		Reference
				Adult	Child	
1-Day HA	114 mg/kg (assumed mouse LOAEL)	single exposure	highest dose not causing death	NA	1.3 mg/ℓ	Hill and Carlisle, 1947
10-Day HA	30 mg/kg (rat NOAEL)	113 days	NOAEL, higher doses caused liver toxicity and depressed growth rate	NA	0.30 mg/ℓ	Rowe and Hymas, 1954
Longer-term HA			Insufficient Data			
Lifetime DWEL	1.0 mg/kg (rat NOAEL)	subchronic exposure*	NOAEL, higher doses caused a variety of effects in blood, liver and kidney indices.	0.35 mg/ℓ	NA	Hazelton Laboratories, 1983

*However, recent findings from the 2-year study confirm the subchronic NOAEL of 1.0 mg/kg/day. Thus, an uncertainty factor of 100 is used to estimate the DWEL rather than the usual 1000.

NA = Not applicable

cell carcinomas (Biometics Research Lab., 1980a). Repeated applications of 2,4-D to the skin of mice only produced papillomas when treatment was preceded by a single dermal application of the initiator 3-methylcholanthrene (Archipov and Kozlova, 1974).

An additonal animal bioassay (Industry Task Force, 1985) conducted by Hazelton Labs in Virginia is available although it has not been critically evaluated by ORD's Carcinogen Assessment Group in this document.

Epidemiology studies (see Subchronic and Chronic Effects in Chapter VI) have associated excess tumor incidence (primarily soft-tissue sarcomas) in humans with mixed exposures to chlorophenoxy herbicides that contain 2,4,5-T (which may be contaminated with 2,3,7,8-TCDD) and 2,4-D (which is not contaminated with this dioxin isomer). Prior to 1986, IARC and EPA have judged the epidemiologic evidence for chlorophenoxy herbicides to be "limited", i.e. providing evidence of causality but not without the possibility of alternative explanations such as chance, bias or confounding factors. The EPA overall weight-of-evidence classification for the chlorophenoxy herbicides is Group B1; IARC (1987) is 2B. While 2,4-D is a member of the chlorophenoxy class and is known to be present as one constituent in some of the chlorophenoxy studies, it is not possible from the studies prior to 1986 to isolate 2,4-D and conclude that it is or is not a causative agent. Thus, prior to 1986, the epidemiology data for 2,4-D alone was judged to be inadequate.

A new case-control study (Hoar et al., 1986), however, is more focused on 2,4-D than the earlier data base. In a carefully designed and well-conducted study, Hoar et al. (1986) found a statistically significant association between exposure to 2,4-D (controlling for the other herbicides present) and an excess risk of non-Hodgkins lymphoma (NHL). The authors also found a dose-related increase of NHL with exposure to 2,4-D.

The CAG believes that the Hoar et al. (1986) study provides evidence of a causal association of 2,4-D exposure with NHL; however, CAG believes that alternative explanations for this association such as chance, bias or confounding factors cannot be excluded. The CAG is aware that the authors of the Hoar et al. (1986) study are currently conducting two additional case-control studies of NHL cases in populations different from those of the Hoar et al. (1986) study. Results from these studies should be available within 12 months. As the Guidelines for Carcinogen Risk Assessment indicate, confidence in inferring a causal association from epidemiologic data is increased when several independent studies are concordant in showing the association. Because the results of the two additional studies will likely be available within 12 months, the CAG has decided to withhold its weight of evidence evaluation of the human data as well as classification of the overall weight of evidence for 2,4-D. CAG's evaluation of the weight of evidence of this chemical will be made pending receipt of the expected epidemiologic studies and a critical evaluation of the Industry Task Force animal bioassay.

Note is made that OPP, by March 23, 1988 Federal Register Notice, has proposed a weight-of-evidence for the cancer data base that takes account of

the Industry Task Force (1985) animal study and the Hoar et al. (1986) epidemiologic data. OPP has proposed that the animal and human data, be viewed as inadequate for 2,4-D, resulting in an over-all weight-of-evidence for 2,4-D of Group D, i.e. data is inadequate to refute or demonstrate a human carcinogenic potential. OPP proposes that additional animal studies be conducted and that a reevaluation of the data base could be initiated at a later date. Public comment on this proposal carries through May 23, 1988.

2,4-D as a commercial product has been shown to contain chlorinated dibenzodioxins as an impurity. A rigorous characterization of the impurities is beyond the scope of this document; however, 2,3,7,8-TCDD and the hexa- isomer have not been detected, while ppb amounts (5-900) have been cited of di-, tri- and 1,3,6,9- or 1,3,6,8-tetra- isomers, depending upon 2,4-D acid %. From a risk characterization perspective, the role, if any, of the impurities warrants recognition as it may pertain to toxicologic observations as well as to the description of the human exposure to 2,4-D, e.g., exposure via drinking water pathway may be to an altered 2,4-D mixture rather than the original commercial preparation, which might not be the case for a pesticide formulator/applicator, for instance.

2,4-D has been tested for mutagenicity in a variety of systems, including microorganisms, plants, fruit flies, cultured mammalian cells, and in vivo mammalian assays (see Mutagenicity in Chapter V). 2,4-D induced mitotic gene conversion and recombination in Saccharomyces cerevisiae (Simmon, 1979); induced recessive lethal and somatic mutations (weakly mutagenic) in Drosophila melanogaster (Magnusson et al., 1977; Rasmusson and Svahlin, 1978); induced mutation to ouabain resistance in cultured Chinese hamster V-79 lung cells and induced unscheduled DNA synthesis in cultured

human fibroblasts (Ahmed et al., 1977); induced chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes (Pilinskaya et al., 1976; Korte and Jalal, 1982); induced bone marrow chromosome aberrations (Pilinskaya et al., 1976); and inhibited thymidine incorporation into testicular DNA (Seiler, 1979) in mice exposed in vivo. Mutagenicity testing of 2,4-D in plants was almost universally positive (see Mutagenicity in Chapter V). A preponderance of negative responses in animal assays, however, indicates that pH may be a critical factor; unless the pH is in the acid range, 2,4-D will be ionized and may not readily cross cell membranes or reach the target tissues. Mutagenicity testing of 2,4-D esters has not been performed, but theoretically these compounds could show higher levels of penetration into target cells. Thus, it may be prudent to expect that these chemicals are mutagenic.

Existing Guidelines, Recommendations and Standards

The National Academy of Sciences has suggested an acceptable level in drinking water of 0.09 mg/ℓ (0.09 ppm) for 2,4-D in drinking water, assuming that 20% of exposure is attributable to drinking water (NAS, 1977). This level was calculated from a NOEL from the Hansen et al. (1971) 2-year feeding study with dogs. The interim primary drinking water standard for 2,4-D is 0.1 mg/ℓ (Federal Register, 1975).

The American Conference of Governmental Industrial Hygienists currently recommends an 8-hour TWA-TLV of 10 mg/m³ for occupational exposure to 2,4-D (ACGIH, 1980). ACGIH also recommends a STEL of 20 mg/m³ for any 15-minute exposure period. These recommendations are intended to protect against local and systemic effects by inhalation and are derived from unspecified ingestion studies.

Occupational exposure limits for 2,4-D have not been recommended by the National Institute for Occupational Safety and Health (NIOSH) or promulgated by the Occupational Safety and Health Administration (OSHA).

The U.S. EPA (1982) has established the following tolerances for 2,4-D residues in or on raw agricultural commodities: 5 ppm for apples, pears, quinces, apricots (includes residues from the preharvest of the dimethylamine salts) and citrus fruits (includes residues from the preharvest application of the isopropyl and butoxyethyl esters and the postharvest application of the alkanolamine salts or the isopropyl ester); and 0.2 ppm for potatoes. Tolerances are established for residues of 2,4-D in acid form, or in the form of several salts and esters on the following commodities: 1000 ppm for rangeland and pasture grasses; 300 ppm for grass hay; 20 ppm for barley, corn, millet straw, oats, rice straw, rye, sorghum, sugarcane and wheat used for forage and fodder; 2 ppm for sugarcane; 0.5 ppm for various grains, cranberries and grapes; and 0.1 ppm for blueberries and rice (U.S. EPA, 1982).

In instances where 2,4-D dimethylamine salt is applied to irrigation ditch banks in the western United States under various Federal programs, the established tolerance for 2,4-D residues is 0.1 ppm for the following commodities: avocadoes, various fruits and vegetables, grain crops, hops, forage grasses and legumes, curcurbits, cottonseed and nuts (U.S. EPA, 1982).

A tolerance for residues of 2,4-D sodium, ethanolamine and isopropanolamine salts calculated as 2,4-D has been established at 5 ppm for asparagus and 0.05 ppm for strawberries (U.S. EPA, 1982).

Established tolerances for residues of 2,4-D are 1 ppm from application of its dimethylamine salt for water hyacinth control in slow moving aquatic media (e.g., western United States irrigation ditch banks) and in fish and shellfish (U.S. EPA, 1982).

Tolerances are established for 2,4-D or its metabolite, 2,4-dichlorophenol, in the following animal food commodities: 2 ppm for cattle, goat, hog, horse and sheep kidney; 0.2 ppm for cattle, goat, hog, horse and sheep meats, meat by-products or fat; 0.1 ppm for milk; and 0.05 ppm in eggs and poultry. A tolerance of 1.0 ppm 2,4-D for residues of its dimethylamine salt or butoxyethanol ester is established for fish in Tennessee Valley Authority dams and reservoirs being controlled for Eurasian Watermilfoil (U.S. EPA, 1982).

A maximum ADI level of 2,4-D for man has been recommended as 0.3 mg/kg by the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues (WHO, 1976), after considering published experimental data and national tolerances established by several countries. An odor threshold for 2,4-D in water was reported by Sigworth (1965) as 3.13 mg/ℓ.

Special Groups at Risk

People who are occupationally exposed to 2,4-D (i.e., agricultural workers and those involved in the manufacture and distribution of the chemical) should be regarded as a special group at risk because they may be exposed to high levels of this chemical. Particularly, there is evidence that humans exposed to chlorophenoxy herbicide formulations containing

mixtures of 2,4-D and 2,4,5-T may develop cancer. Toxicokinetic studies with humans indicates that considerable interindividual variation occurs in the rates of absorption and excretion and in the amount of 2,4-D conjugated; these differences might result in a wide range of sensitivity to 2,4-D among individuals. Pregnant women should also be regarded as a sensitive population because 2,4-D and some of its salts and esters have produced fetotoxic and developmental effects in experimental animals. Because 2,4-D is excreted primarily in the urine and has some toxicity to the kidneys, persons with renal disease would also be a special group at risk.

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