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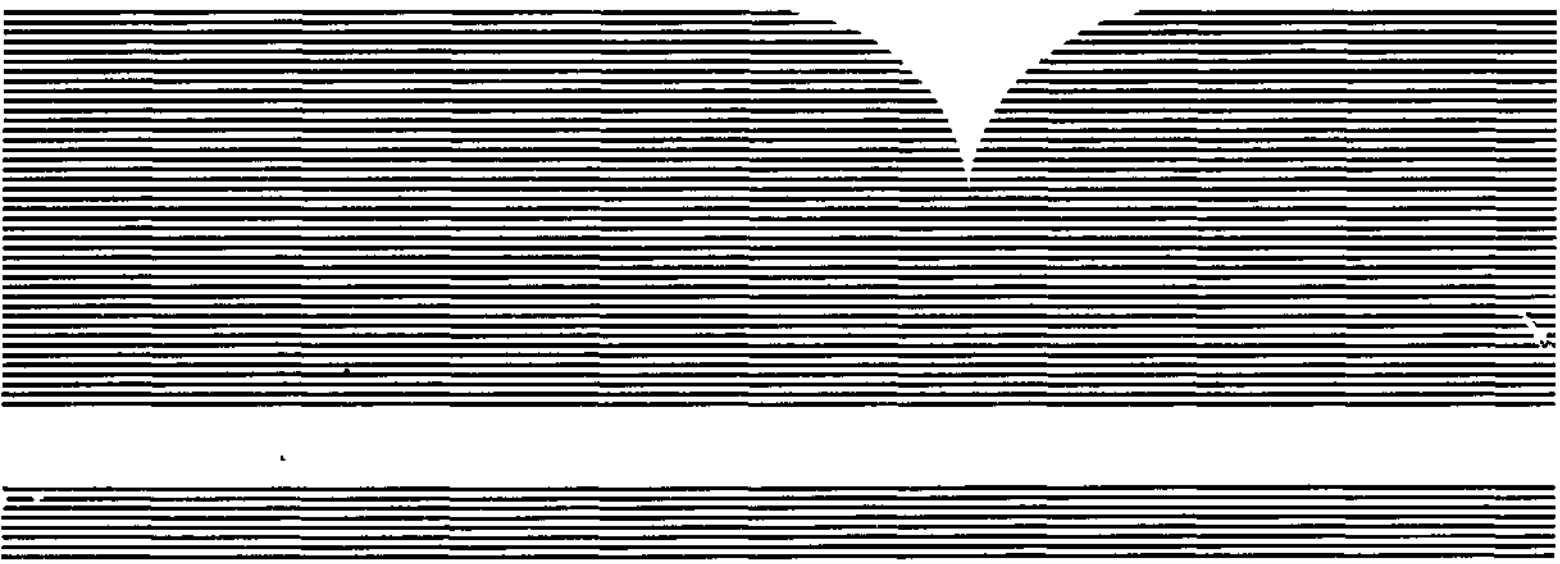

U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

2,4,5-T: Position Document 1

EPA/SPRD -80/76

2,4,5-T Working Group
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2,4,5-T: Position Document 1

2,4,5-T: POSITION DOCUMENT 1

I. BACKGROUND

A. Chemical/Physical Characteristics

The herbicide commonly known as 2,4,5-T (chemical name, 2,4,5-Trichlorophenoxyacetic Acid) has an empirical formula of $C_8H_5Cl_3O_3$. The pure acid form occurs as white crystals and has a molecular weight of 255.49. The melting point is 156.6°C. Its solubility in water is 278 parts per million (ppm) at 25°C; it is also soluble in acetone, ethanol, ether, and alkaline solutions (1). The esters of 2,4,5-T are formulated to be emulsifiable in water and soluble in most oils, while its amine salts are soluble in water but insoluble in petroleum oils (2, 3).

B. Manufacturing Process and Contaminants

2,4,5-T is produced commercially by a process using 1,2,4,5-tetrachlorobenzene as the starting material which is reacted with methanol and sodium hydroxide under high temperature and high pressure to give the sodium salt of 2,4,5-trichlorophenol (2,4,5-TCP).[1/]

1/ 2,4,5-TCP is the subject of a separate Rebuttable Presumption Against Registration (RPAR) Position Document. It is discussed in this document because both it and its contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may be present in some commercial 2,4,5-T and in 2,4,5-T samples used in animal experiments.

This product is reacted with chloroacetic acid under mildly alkaline conditions. Sulfuric acid (H_2SO_4) is then added to the product of this step to produce 2,4,5-T. The acid form of 2,4,5-T can be readily reacted with a variety of alcohols to produce a large selection of esters and with amines to produce amine salts (3).

During the first step in the manufacturing process of 2,4,5-T, if temperature and pressure are not carefully controlled, highly toxic contaminants, polychlorinated dibenzo-p-dioxins, may be formed in large quantities. The particular dioxin formed is dependent on the chlorophenols present (4). The term dioxin does not apply to any one compound but to a group of related substances, which are distinguished by the number and orientation of chlorine atoms they contain. Dioxin toxicity also varies with the position and numbers of chlorines attached to the phenol rings.

In the 2,4,5-T manufacturing process an especially toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is formed when the reaction temperature is excessive (8, 9, 10, 11, 12), most commonly at temperatures above 160°C. Halogens at the 2, 3, and 7 positions are known to produce toxic dioxins (13). In the case of TCDD, the chlorine atoms

are attached at the 2, 3, 7, and 8 positions which are considered the most toxic positions possible (14). The dioxin contaminant in 2,4,5-T is of particular concern because of its extremely high toxicity, and because of the apparent inability of manufacturers to produce 2,4,5-T without the contaminant, TCDD (7).[2/]

TCDD occurs as a white crystalline solid. It is 99.5% decomposed at 800°C. TCDD has the following solubility in various solvents at 25°C (7).

Solvent	Solubility (wt. per cent)
Acetone	0.011
Benzene	0.057
Dimethylsulfoxide	<0.01
Methanol	0.001
Water	0.00000002 (0.2 ppb)

It has been recognized for quite some time that chlorinated dibenzo-p-dioxins occur as possible byproducts (contaminants) in the manufacturing of chlorinated phenols (15). The formation of TCDD during production of 2,4,5-TCP was demonstrated by Kimmig and Schulz (16). TCDD was obtained from the pyrolyzing of 2,4,5-TCP by Higginbotham

2/ Since manufacturers are unable to produce 2,4,5-T without TCDD, all references to 2,4,5-T in this document refer to 2,4,5-T contaminated with some level of TCDD.

et al. (11). They noted that the specific dioxin formed depended on the chlorophenol pyrolyzed. Kearney et al. (17), however, reported that TCDD is historically associated with any pesticide derived from 2,4,5-TCP. A number of researchers (12, 18, 19, 20, 21) have reported on the formation of TCDD by thermal decomposition of the sodium salt of 2,4,5-TCP under alkaline conditions during the manufacturing process.

Since 1950, most of the chemical industry has known that large quantities of TCDD may be formed as a byproduct of the 2,4,5-TCP manufacturing process if the procedures are not carefully controlled. At one time 2,4,5-T was produced which contained between 30 to 40 ppm of TCDD (7, 22, 55). Between 1968 and 1969, one manufacturer had a 90% decrease in the amount of TCDD present in the 2,4,5-T it produced. Different manufacturers produced 2,4,5-T with different TCDD contents (17).

After concern arose in 1969 about the extremely toxic effects of TCDD, manufacturing methods were changed and carefully controlled by manufacturers. By 1971 industry had reduced TCDD content in commercial samples of 2,4,5-T to less than 1 ppm (9, 23, 24). Current U.S. manufacturing specifications require 2,4,5-T presently being sold to

contain less than 0.1 ppm TCDD (7). Several countries now produce commercial 2,4,5-T containing less than 0.05 ppm TCDD (25).

C. Formulation and Class

2,4,5-T is classed and used as a selective herbicide, especially for brush control (2). It is formulated in many forms of salts and esters which are available as emulsifiable concentrates containing 2, 4, or 6 pounds actual acid equivalent per gallon and as oil soluble concentrates with 4 or 6 pounds active ingredient (AI) per gallon. The most commonly used formulations are the low volatile esters (26). 2,4,5-T also occurs in registrations mixed with 2,4-D, Dicamba, Picloram, Silvex, and 2-(2-methyl-4-chlorophenoxy) propionic acid (27).

D. Registered Uses and Production

2,4,5-T has been produced as a registered pesticide in the United States since 1948. According to EPA records, approximately 122 companies hold Federal registrations and formulate 424 registered products; eleven companies have former state registrations[3/] and formulate 21 products.

3/ Pesticide products formerly registered under state pesticide registration laws and shipped or distributed for sale solely within intrastate commerce are subject to Federal pesticide regulations under 40 CFR Section 162.17(a). Application has been made to obtain Federal registration for intrastate use of these products. For a list of trade names under which 2,4,5-T is marketed, see the registrant/product list attached to this document.

Section 7(c) of FIFRA requires manufacturers and formulators to submit to EPA information on production, sales, and distribution. Under FIFRA sections 7(c) and 10, this information may not be made available to the public. A confidential memorandum containing this information has been sent to the Deputy Assistant Administrator for Pesticides (28). The Pesticide Review (29) reported that 11,626,000 pounds of 2,4,5-T acid, esters, and salts were produced in the United States in 1969 and 12,335,000 pounds in 1970. The Pesticide Review (29) also reported that the United States imported 738,907 pounds of 2,4,5-T during 1971 through 1974.4/ Of this total 155,342 pounds were imported in 1974. This was down from nearly 392,000 pounds in 1973 but up from the 5-year average of 148,000 pounds. While The Pesticide Review (29) does not report export figures for 2,4,5-T alone, it does report exports of 2,4-D and 2,4,5-T together. Export of 2,4-D and 2,4,5-T was reported at 6.8 million pounds in 1972; 21 million pounds in 1973; and almost 22 million pounds in 1974.

A great deal of variability exists in reports on usage of 2,4,5-T. Agricultural end-use data obtained from the National Study of Agricultural, Governmental, and Industrial Uses of Pesticides, conducted by this Agency (30), indicated the following uses of 2,4,5-T in the United States in 1974.

4/ The level of TCDD in the imported 2,4,5-T was not reported.

Crop	pounds AI applied	% total agriculture
Rangeland and Pastures	968,000	97.24
Rice[a/]	16,000	1.54
Nursery Crop	12,000	1.20
Turf and Ornamentals	200	0.02
Blueberries[b/]	8	--
Estimated total use in agriculture for 1974	996,000	100.00

a/ The Agency has looked at effects on aquatic organisms representative of species likely to be exposed from application of the triethylamine formulation of 2,4,5-T to rice. The calculated concentration of this formulation in a 6-inch layer of water at the highest recommended use rate is 0.9 ppm. The LC-50 bioassay values for bluegill and catfish are well above this level (ranging from a 24-hour LC-50 of 53 ppm for bluegill to a 96-hour LC-50 of >72 ppm for bluegill and channel catfish). Rainbow trout, which cannot be considered "representative of the organisms likely to be exposed" in the geographic areas where rice is grown, have a 96-hour LC-50 ranging from 0.7 to 0.07 ppm.
b/ This is no longer a registered use.

In addition, this survey reported that 324,491 pounds of active 2,4,5-T were used by federal and state agencies and 659,463 pounds by industry.

Other sources have reported usages for 1974 as follow: rights-of-way, 4 million pounds; rangeland, 1.5 to 2.3 million pounds; rice, 220,000 pounds; and forestry, 50,000 pounds.

E. Metabolism in Experimental Systems

Several studies have demonstrated that 2,4,5-TCP is the primary degradation product or metabolite formed in the breakdown of 2,4,5-T, by either physical or biological

mechanisms. Crosby and Wong (31) found that 2,4,5-TCP was one of the major decomposition products in the photodecomposition of 2,4,5-T in water. Sharpee (32) found that microbial degradation of 2,4,5-T in culture, soil, and aquatic ecosystems resulted in the formation of small amounts of 2,4,5-TCP.

Shafik et al. (33) dosed Sprague-Dawley rats by gavage with 2,4,5-T at 50, 5, 0.05, and 0.005 mg/kg for three days. Two rats were dosed at each level. The authors found that, at 0.005 mg/kg, excretion of 2,4,5-T in urine was complete two days after the final dose. They also found 2,4,5-TCP excreted as a metabolite in the urine of rats given 50 mg/kg, but no detectable 2,4,5-TCP was found at the two lowest dose levels. A hydroxylated trichlorophenoxyacetic acid and a hydroxylated trichlorophenol were identified, by unconfirmed mass spectrometric analysis, as possibly being two additional metabolites of 2,4,5-T.

Grunow et al. (34) studied seven male Wistar rats fed a single 2,4,5-T dose at 50 mg/kg body weight. They found that the daily renal excretion of free 2,4,5-T was, in general, at its maximum on the second day after feeding. After seven days, free 2,4,5-T in the urine decreased to a value below 2% for all animals. In addition to 2,4,5-T excreted in the free form, the authors found it to be excreted as derivatives which could be converted into 2,4,5-T by acid hydrolysis. They were able to identify one of these as N(2,4,5-trichlorophenoxyacetyl) glycine.

Grunow and Bohme (35), in a study using Wistar rats and NMRI mice, fed doses of 2,4,5-T at 200 mg/kg body weight. These authors isolated N(2,4,5-trichlorophenoxy

acetyl) taurine as a metabolite of 2,4,5-T, in addition to the metabolites named above.

Clark et al. (36) found residues of 2,4,5-TCP in the muscle, liver, and kidney of sheep which were fed rations containing 2,000 ppm of 2,4,5-T for 28 days. The 2,4,5-T used in this study had a purity of 99% and contained no detectable dioxin (detection limit: 0.5 ppm).

Leng (37) conducted a feeding study during 1969 and 1970, in which dairy and beef cattle and sheep were given 2,4,5-T at levels from 10 to 2,000 ppm in the total diet for intervals of two to four weeks at each level tested. The author reported that no residues (<0.05 ppm) occurred in milk or cream of cows ingesting 10 to 30 ppm 2,4,5-T. At 100 ppm 2,4,5-T in the diet, traces of 2,4,5-TCP (0.06 ppm) appeared in milk and cream. When given high levels of 2,4,5-T, equivalent to 300 and 1,000 ppm in the total diet, residues of 2,4,5-T and 2,4,5-TCP ranged from 0.05 to 0.5 ppm in the milk of individual cows.

Fitzgerald et al. (38), studying the degradation of 2,4,5-T in woody plants, reported that colorimetric analysis suggested, and chromatographic analyses confirmed, that the n-butyl ester of 2,4,5-T is degraded in sweet gum (Liquidambar styraciflua) and southern red oak (Quercus falcata) to yield 2,4,5-TCP.

F. Environmental Fate

(1) Persistence: Soils

Soil surface and foliage are the major recipients of phenoxy herbicides (39) whether applied by ground spray systems or from aircraft. Once 2,4,5-T reaches the soil it may be degraded chemically or biologically, volatilized and moved to other areas, absorbed on soil colloids or in organic matter, or leached to depths or locations where it cannot be absorbed by plant roots (47).

Norris et al. (176) reported on the persistence of 2,4,5-T in a Pacific Northwest forest. The authors found that six months after application of 2,4,5-T at 2.24 kg/ha (2 pounds/acre), the level of herbicide in the forest floor declined 90%; after one year, less than 0.02 kg/ha remained in the forest floor. The authors found little leaching of 2,4,5-T from the forest floor into soil, and no residues were found deeper than 15 cm (maximum residue found was 0.08 ppm) despite rainfall of 24 cm the first month and 70 cm the first three months after application. Norris et al. (176) stated that the rapid disappearance of 2,4,5-T from the forest floor suggests abundant microbial activity. Norris (40) reported microbial activity to be important in the disappearance of 2,4,5-T from forest-floor material in the laboratory.

Wiese and Davis (41) found that, in an agricultural soil, 2,4,5-T remained in the upper six inches even after application of 4.5 inches of water over a short period of time.

Helling et al. (39) found that 2,4,5-T is relatively mobile in sandy soils but that movement decreases as organic content increases. Thus 2,4,5-T is moderately mobile in clay soils and only slightly mobile in muck (42).

Yoshido and Castro (43) studied the degradation of 2,4-D, 2,4,5-T, and Picloram in two Philippine soils under upland and submerged conditions. The authors found the degradation of 2,4,5-T to be rapid in Maahas clay. Slightly more 2,4,5-T residues were recovered in submerged than in upland Maahas soil. In Luisiana soil under submerged conditions, 2,4,5-T degraded rapidly in eight weeks after a four-week lag period, while it degraded gradually under upland conditions, with only about 40% of the 2,4,5-T recovered after 12 weeks.

Morton et al. (44), using technical grade 2,4,5-T labeled in the carboxyl position with carbon-14, found that its apparent half-life averaged 1.6 weeks in green tissues of native grasses at College Station and Spur, Texas, and 1.7 weeks in litter tissue. The authors stated that the

amount and frequency of rainfall were conducive both to leaching and microbial decomposition of the herbicide, and to growth of sideoats gramma plants, all of which were factors contributing to rapid reduction of herbicide concentrations.

When considering the persistence of 2,4,5-T, the persistence of its manufacturing contaminant, TCDD, must also be considered. Helling et al. (39) found that TCDD was not photodecomposed on soil. TCDD was found to be immobile in Norfolk and Lakeland sandy loams, Hagerstown silty clay loam, Barnes clay loam, and Celeryville muck, and was not leached further into soil by rainfall or irrigation. During surface erosion of soil, however, lateral transport of TCDD could occur.

The persistence of TCDD in Lakeland loamy sand and Hagerstown silty clay loam at 1, 10, and 100 ppm was studied by Kearney et al. (46) for 360 days. After one year these researchers recovered 56 and 63% of the originally applied TCDD in Hagerstown and Lakeland soils, respectively. Helling et al. (39) observed that TCDD's persistence was predictable since it is insoluble in water.

(2) Persistence: Water

Current information indicates that, although some 2,4,5-T may enter streams flowing through or adjacent to

areas being sprayed, residue levels in streams will be very low. Norris (47) reported the results of an intensive study of stream contamination from spray projects on range and forest lands in Oregon which showed that peak concentrations of phenoxy herbicides seldom exceeded 0.1 ppm and that herbicide residues persisted for only a few hours in nearly all streams. Norris (47) speculated, however, that application of herbicides to marshy areas may result in high-level, long persistence of chemical residues in nearby streams.

The Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency (48) stated that all available data indicated that the amount of 2,4,5-T entering water is small and does not persist long. It is adsorbed on clay or absorbed by biota within a matter of days.

Phenoxy chemicals entering water may be lost by volatilization, degradation, adsorption on sediment, adsorption by biota, and dilution as additional stream water passes through the site. Almost all authorities agree that there is adsorption on bottom sediment (48, 49, 50).

Kenaga (51) stated that esters of 2,4,5-T in most kinds of water, except highly acidic waters, are usually hydrolyzed within a matter of days. When the 2-ethylhexyl

ester of 2,4,5-T was applied to water in the laboratory at a concentration of 1 ppm for an hydrolysis study, 58% remained after 4 hours; 33% after 8 hours; and 12% after 16 hours.

Trichell et al. (52), studying the loss of herbicides in runoff water, found 2 ug/ml of 2,4,5-T in runoff water 24 hours after it was applied at 2.24 kg/ha, after which 1.3 cm of rainfall was simulated on sod-covered plots of 3% slope. Four months after application, concentrations of 2,4,5-T in runoff water had diminished to 0.04 ug/ml.

Edwards and Glass (53) monitored runoff and percolation of 2,4,5-T at Coshocton, Ohio, for 14 months following application of 11.2 kg/ha of 2,4,5-T and found that 5.5 g/ha, or over 0.05% of the herbicide, was lost from the treated area. Most of the 2,4,5-T was removed in runoff water during the first four months after application, and more than half of the loss occurred the first month after treatment.

Kearney et al. (46) concluded that contamination of underground water supplies with TCDD seemed very unlikely, since vertical movement of TCDD did not occur in a wide range of soil types. The fact that no leaching occurred, however, would not preclude runoff loss when soil erosion is significant (39).

(3) Transport

Isensee and Jones (54) measured uptake of TCDD from soil by two crop species. Oats (Avena sativa) and soybeans (Glycine max) were grown in Lakeland sandy loam soil treated with 0.06 ppm TCDD. The concentration of TCDD in soil was approximately 4,000 times greater than the amount that would be deposited in soil from an application of 2,4,5-T (with 1 ppm TCDD) at a rate of 2 pounds/acre in the top 1/3 inch of the soil surface. The tops of these plants were harvested at intervals to maturity. Mature oats and soybean tops contained less than 1 part per billion (ppb) TCDD. TCDD was detected (with a detection limit of 1 ppb) in mature oat grain, while no TCDD was found in the bean of soybeans. The authors concluded that soil uptake of TCDD by plants was highly unlikely, since little or no TCDD was taken up by oats or soybeans under the conditions of this experiment (54).

(4) Bioaccumulation

Woolson et al. (55) conducted a study to determine if TCDD residues could be detected in bald eagle (Haliaeetas leucocephalus) tissue extracts, as a representative of the top of a food chain. Scientists at the Patuxent Wildlife Center (U.S. Department of the Interior, Laurel, Maryland) collected, and furnished to these researchers, 19 bald eagle

carcasses from Alaska, Maine, North Dakota, Wisconsin, Michigan, Minnesota, Arkansas, Illinois, Missouri, Maryland, Virginia, Iowa, New York, New Jersey, and Florida between 1966 and 1971. These states were selected as sampling sites in order to provide a widely dispersed sample population. The eagle tissues were prepared and extracted as described by Mulhearn et al. (56). Woolson et al. (55) detected no dioxin residues at a level of 0.05 ppm TCDD, the lower limit of detection for most pesticides in tissue samples run by the Patuxent Wildlife Research Center at that time. The authors stated that the non-detection of dioxin residues could imply that there was no dioxin build-up in the food chain; that the build-up was less than the [then] current detectable level of 0.05 ppm [50 ppb]; that the eagles examined were not contaminated although other samples might be; or that other species could feed on a different food chain to accumulate dioxins.

Isensee and Jones (57) exposed several organisms in a model aquatic ecosystem to ^{14}C-labeled TCDD for up to 31 days to determine the distribution and bioacumulation potential in the aquatic environment. Soil containing from 0.0001 to 7.45 ppm adsorbed ^{14}C-TCDD was placed in aquaria, containing eight snails (Physa sp.), a few strands of algae (Oedogonium cardiacum), and 10 ml of old aquarium water

containing various diatoms, protozoa, and rotifers. Fifteen duckweed (Lemna minor) plants were also added to one aquarium. Samples of daphnids were taken for analysis at 30 days, and two mosquito fish (Gambusia affinis) were added to each tank. Three days later all of the organisms were removed for analysis, and two fingerling channel catfish (Ictalurus punctatus) were added to each tank and exposed for six days.

The authors stated that all organisms in both treatment and control tanks prospered during this exposure period, indicating that TCDD was not toxic at the concentrations used. TCDD accumulated in all organisms. At the highest TCDD concentration (7.45 ppm) algae accumulated 6,690 ± 960 ppb TCDD; snails, 1,820 ± 170 ppb; daphnids, 10,400 ± 480 ppb; and Gambusia, 1,380 ± 220 ppb. Catfish were not analyzed for TCDD residues. At the second highest TCDD concentration (3.17 ppm), however, catfish accumulated 720 ± 130 ppb TCDD. The authors stated that accumulation in all of the test organisms from soil containing 0.1 ppb TCDD is important since this concentration approaches the concentration which would occur under normal field use of 2,4,5-T. The authors concluded that the data suggested that under certain circumstances (discharge of storm runoff from

recently treated rangeland into a small pond), water-eroded surface soil or debris may contain enough TCDD for measurable residues (parts per thousand [ppt] quantities) to accumulate in fish or other aquatic organisms. However, the authors speculated that TCDD, orginating from 2,4,5-T applications, discharged into large lakes, streams, or estuaries would probably become sufficiently diluted so that no measurable accumulation would occur.

As part of a broad study to determine whether 2,4,5-T use leads to TCDD accumulation in the environment, Shadoff et al. (58) collected samples of fish, mud, water, and human milk from areas in Texas and Arkansas. The Texas samples of water, mud, catfish, and walleyed pike were collected from the San Angelo Reservoir, an impoundment of the North Concho River. The authors stated that this watershed has large acreages that have been sprayed with 2,4,5-T at 0.5 pounds/acre (2,4,5-T acid equivalent) for brush control. These researchers also obtained six samples of human milk from mothers residing in the general area of the San Angelo Reservoir. In addition, bass from a 125-acre pond in the heart of the Arkansas rice-growing area were collected. Water from this pond is used to flood rice fields treated with the equivalent of 1.25 pounds/acre of 2,4,5-T acid, four to eight weeks prior to flooding. The water is

later drawn off the fields and pumped back into the pond for re-use. In addition, the pond is supplemented by water from wells and by water collected as run-off from surrounding rice fields during the rainy season. The authors stated that this cycle had been in use (including the proper use of 2,4,5-T) for 18 years up to the time of their study. The authors stated that no TCDD was detected in the tissues sampled, using a Gas Chromatography-Mass Spectrometry procedure with a detection limit which averaged less than 10 ppt. No evidence was found that TCDD is accumulating in the environment from the use of 2,4,5-T described in this study.

G. Residues

(1) Soil

Woolson et al. (55) studied Lakeland sandy soil to determine if TCDD residues could be detected in soil receiving exceedingly large application of 2,4-D and 2,4,5-T. The heaviest rate of 2,4,5-T application was 947 pounds/acre applied aerially during 1962 through 1964, while the lightest rate was 160 pounds/acre applied aerially during 1968 and 1969. During this period, it was not uncommon for commercial samples of 2,4,5-T to contain levels of 30 to 40 ppm TCDD.

The authors were able to detect small amounts of 2,4,5-T in the soil samples. They observed that the residue level decreased with time after application and stated that leaching and microbial decomposition could account for this decrease. Using a detection limit of less than 1 ppb, the authors did not detect any TCDD at any depth in 36-inch core samples of the soil.

(2) Water

In October 1965, the U.S. Geological Survey initiated a limited program of pesticide monitoring on 11 waterways in the western United States (59). The streams, representing agricultural areas where the probability of observing pesticide residues would be greater, included the Missouri, Brazos, Yellowstone, Sacramento, Colorado, Arkansas, Yakima, Rio Grande, and Snake Rivers. Pesticides chosen for analysis included the insecticides aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane, and the herbicides 2,4-D, 2,4,5-T, and silvex. The authors reported that no herbicide was found at any time at any station during the first year of the sampling program. The lower limit of sensitivity (detection) was 5 ppt.

Manigold and Schulze (60), reporting on the results of the U.S. Geological Survey stream monitoring program for

the two-year period October 1966 to September 1968, observed that beginning in August 1967 2,4-D, silvex, and 2,4,5-T had been detected frequently. 2,4,5-T was found in 28 of the 320 samples and ranged from 0.01 to 0.07 ppb. The authors stated that the established criteria permitted 100 ug/liter (ppb) for herbicides. These authors reported that the analytical procedures were changed from the preceding report to use Law's sample clean-up procedure, which permits routine detection of pesticides at 0.005 ug/liter in most waters.

Norris (47) observed that peak concentrations of phenoxy herbicides seldom exceeded 0.1 ppm in streams contaminated from spray projects on range and forest lands in Oregon.

Lawson (61) studied 2,4,5-T residues in storm runoff from three small watersheds in Arkansas. Two watersheds, one cleared and the other partially cut, were sprayed with the isooctyl ester of 2,4,5-T. A third watershed, adjacent to the two treated ones, was used as a control. Spraying was done in September 1971, June 1972, and July 1973, either to control woody sprouts and broadleaf vegetation or just to provide herbicide application for monitoring. The cleared watershed was treated with 4 pounds acid equivalent per acre and the partially cut site with 2 pounds/acre.

In water samples taken after the first runoff-producing storm in October 1971, Lawson (61) detected an average of 2.1 ppm 2,4,5-T from the cleared watershed and 1.0 ppm from the partially cut site. Maximum amounts detected were 2.2 and 1.3 ppm for the two areas. No 2,4,5-T was detected from the control site.

Only trace amounts (less than 0.2 ppm) were detected from each of the two treated sites after the next runoff-producing storms in November 1971. None was detected from the control.

In approximately 90 samples taken after storms during the period December 1971 through September 1973, no 2,4,5-T was detected by Lawson (61) in the runoff from the treated or control water sheds.

Since TCDD is immobile in soil (39) and soluble in water at only 0.2 ppb (7), the possibility of ground water contamination is virtually nonexistent (46). TCDD could be present in runoff when soil erosion is significant (39), and thus TCDD contamination of water bodies could occur.

A recent National Academy of Sciences report on drinking water stated that 2,4,5-T and TCDD have never been detected in drinking water; the limit of detection was in

the parts per trillion. However, the report did project the toxicity of 2,4,5-T and TCDD, their acceptable daily intake, and suggested no-adverse-effect levels (62).

(3) Air

Prior to 1970, phenoxy herbicides were widely used for early postemergence control of weeds in wheat. Johnson (63) reported that air samples collected during spring and summer in the state of Washington where these crops are grown contained as much as 0.06 ug/m^3 2,4-D and 2,4,5-T. Assuming that a man inhales about 30 m^3 of air per day, the authors estimated that exposure to 0.06 ug/m^3 would amount to inhalation of 1.8 ug phenoxy herbicide/day or 0.025 ug/kg of body weight per day for a 70 kg man.

Ambient air monitoring for pesticides in predominantly agricultural areas of 28 states was conducted by the National Air Monitoring Program in calendar years (CY) 1970 through 1972 using ethylene glycol impinger type samples. Table 1 records the arithmetic mean of residues of 2,4,5-T detected in this program (64).

Table 1. Air Monitoring Data for 2,4,5-T in 28 State Monitoring Programs (1970 to 1972)

Name of State or City	2,4,5-T Ester Monitored For	ng/m^3 CY 1970	ng/m^3 CY 1971	ng/m^3 CY 1972
Louisana	Isopropyl ester	-	ND	1.9
Montana		ND	ND	0.8
New Mexico		-	ND	1.0
Idaho		ND	ND	1.7
Illinois	BOEE	ND	3.6	ND
Oregon		ND	0.5	ND
Tennessee		1.1	ND	ND
Tennessee	Isooctyl ester	ND	2.7	ND
Oklahoma		ND	14.6	ND

ND = Not Detected.

(4) Animals

Phenoxy acetic acids are relatively strong acids, and animals rapidly excrete them unchanged in their urine (36). In their study of the fate of atrazine, kuron, silvex, and 2,4,5-T in the dairy cow, St. John et al. (65) found that dairy cows given 2,4,5-T and silvex in their feed at 5 ppm for four days, completely eliminated both 2,4,5-T and silvex as soluble salts in the urine two days after dosing stopped.

Zielinski and Fishbein (66) treated female C57BL/6 mice with a single subcutaneous injection of 100 mg/kg body weight of 2,4,5-T in dimethylsulfoxide solution. They sacrificed the animals at various intervals after injection and analyzed *in toto* for 2,4,5-T. The amounts recovered as percentage of the amount injected indicated decreasing levels at the following time intervals after dosing: at 0 hours, 77.1 ± 5.0%; at 16 hours, 56.9 ± 4.2%; and at 24 hours 23.7 ± 3.6%.

In a preliminary report of a two-year chronic toxicity feeding study, Dow Chemical USA (110) reported the following residue data for rats fed indicated TCDD doses: 24,000 ppt in liver and 8,100 ppt in fat of females ingesting 2,200 ppt/day; 5,100 ppt in liver and 1,700 ppt in fat of females ingesting 220 ppt/day; and 540 ppt in liver and fat of females ingesting 22 ppt/day. The preliminary report gives no residue data for treated males, or for controls of either sex.

Piper et al. (67) studied the fate of 2,4,5-T following oral administration to rats and dogs. Four groups of three male and three female Sprague-Dawley rats (Spartan strain) and two male and two female adult beagle dogs were given single doses of ^{14}C-labeled 2,4,5-T by intubation at 5, 50, 100, and 200 mg/kg body weight in rats and 5 mg/kg body weight in dogs. The authors combined data obtained for males and females since the pharmacokinetics of 2,4,5-T were essentially the same in each sex. In this study, the clearance half-life for 5 mg/kg 2,4,5-T from dog plasma was 77.0 hours; in rats the half-life was 4.7 hours at 5 mg/kg and 4.2 hours at 50 mg/kg. At doses of 100 and 200 mg/kg body weight, the clearance half-life for rats increased to 19.4 and 25.2 hours, showing that the

pharmacokinetics of 2,4,5-T varies with dose as well as with species. The authors suggested that the half-life values at 100 and 200 mg/kg body weight indicated that these doses may have exceeded the excretory capacity of the rats.

Zitko (68) assayed chlorinated dibenzodioxin residues in aquatic animals, but was unable to detect these compounds (detection limit: 0.04 ug/g [ppm] for TCDD) in any of several aquatic animals from Canadian locations. The author had selected species from high trophic levels of the aquatic food web to measure cumulative pesticide contamination. More recently, using improved analytical methods for detection of dioxin at ppt levels, Baughman and Meselson (69) found mean TCDD levels ranging from 18 ppt to 810 ppt in fish and crustaceans taken from Vietnamese rivers in August and September 1970. TCDD levels tended to be higher in fish from interior rivers than in those from seacoast locations. In comparison, Baughman and Meselson found less than 3 ppt TCDD in fish obtained in a market in Cape Cod, Massachusetts. In another study, Matsumura and Benezet (70) placed TCDD-coated sand directly in an aquarium containing brine shrimp, mosquito larvae, and fish (silverside). TCDD pickup was low in fish (2 ppb) and brine shrimp (157 ppb) under the experimental conditions. But mosquito larvae, which are bottom

feeders, showed a surprisingly high rate of pickup (4,150 ppb). The authors concluded that TCDD was not likely to accumulate in as many biological systems as DDT because of TCDD's low solubility in water and lipids, as well as its low partition coefficient in lipids.

(5) Plants

Clark et al. (36) reported that, when herbicides are applied to rangeland, the levels of phenoxy herbicides available for ingestion by grazing livestock depend upon the nature and degree of cover, the rate and mode of application, time after application, and climate conditions. Studies by Morton et al. (44) showed that residues on grass immediately after application of 2,4,5-T are not likely to exceed 100 to 150 ppm for each pound of actual herbicide applied per acre.

Leng (37) stated that herbicide residues in or on plants declined rapidly, with a half-life of one to two weeks, due to photodecomposition by sunlight, wash-off by rain, metabolism by plants, and dilution from growth of plants. 2,4,5-T was applied to grass in four states at an application rate of 4 pounds/gallon, 3 gallons/acre; initial residues immediately after treatment in California averaged 684 ppm (or 57 ppm/pound applied per acre); 1,668

ppm (or 139 ppm/pound) in Michigan; 1,464 ppm (or 122 ppm/pound) in North Carolina; and 1,332 ppm or (111 ppm/pound) in Texas. After two weeks, residues in the four locations averaged 26 to 34 ppm/pound per acre. After 16 weeks, all residues had declined to an average 3 ppm/pound applied per acre.

Baur et al. (71) treated grass species indigenous to Victoria County, Texas, with 2 pounds/acre 2,4,5-T ester. One month after application the concentration averaged 4,060 ng/g (ppb) for 2,4,5-T acid and 2,890 ng/g (ppb) for 2,4,5-T ester. Six months after application the concentration averaged 60 and 170 ng/g (ppb) for 2,4,5-T acid and ester, respectively.

Getzendaner and Hummel (72)[5/] described a 1969 study in which a 2,4,5-T propylene glycol butyl ether ester formulation was sprayed on Texas grass at an application rate equivalent to 12 pounds of 2,4,5-T per acre; this rate was 6 to 24 times the usual rate applied to grazing lands for brush control. At this time, manufacturing specifications

5/ Studies submitted by registrants as part of petitions for residue tolerances are classified confidential, pending outcome of litigation in U.S. District Court.

for no detectable TCDD in 2,4,5-T used a method sensitive to 1 ppm. The authors found that residues of TCDD decreased rapidly from about 500 ppt TCDD within one day of application, to about 35 ppt TCDD after four weeks, and about 15 ppt TCDD after 16 weeks. The TCDD decrease roughly paralleled the loss of 2,4,5-T from the same grass.

(6) Humans

Matsumura (73) studied 2,4,5-T in the blood and urine of human male volunteers who had ingested the chemical. After ingesting 150 mg (2.2 mg/kg), the plasma concentration of 2,4,5-T in one subject reached a peak of 21.1 ug/ml after four hours. A linear, semi-logarithmic concentration-time curve (a gradient of -0.065) four hours post-treatment indicated first order elimination and absorption kinetics.

In a second part of this study, Matsumura gave two male volunteers single oral doses of 100 mg 2,4,5-T. Urine samples were collected over 72 hours. About 45% of the original dose was found in urine collected during the first 24 hours after treatment; 60% had been recovered 36 hours after treatment; and after 72 hours, more than 80% of the original dose of 2,4,5-T had been recovered.

Gehring et al. (74) also studied the fate of 2,4,5-T following oral administration to man. Five male volunteers,

ages 31 to 58 years, each ingested a single 5 mg/kg oral dose of analytical grade 2,4,5-T, with a purity greater than 99% and less than the detectable level (0.05 ppm) TCDD, directly or as a slurry in milk. Blood, urine, and feces were collected at intervals for up to 96 hours after ingestion. Essentially all (88.5 ± 5.1%) of the 2,4,5-T ingested by these subjects was excreted unchanged in the urine after 96 hours. The plasma 2,4,5-T concentration increased rapidly following ingestion and after 7 hours reached a peak of approximately 57 ug/ml, after which the plasma contained 65% of the 2,4,5-T in the body, of which 99% was bound reversibly to protein.

Kohli et al. (75) also studied absorption and excretion of 2,4,5-T in man. Eight male volunteers, age 25 to 35 years, received a single oral dose (2, 3, or 5 mg/kg) analytical grade 2,4,5-T with a purity greater than 99%. Urine was collected up to 96 hours, and blood samples were collected up to 168 hours. 2,4,5-T was detected in some two-hour urine samples, indicating rapid excretion of the compound. More than half of the 2,4,5-T was excreted in the urine in the first 48 hours, although small quantities were still being excreted at 96 hours.

2,4,5-T appeared in all plasma samples one hour after 2,4,5-T ingestion, indicating rapid absorption. Maximum concentration (approximately 25 ug/ml for the 5 mg/kg dose) was reached between 7 and 24 hours after ingestion and began to decline at a first-order rate after 32 hours.

These investigators concluded that 2,4,5-T was readily absorbed from the gastrointestinal tract, that it was eliminated unchanged in the urine, and that the half-life for plasma clearance was 18.8 ± 3.1 hours. These authors pointed out that, in general, higher recoveries were reported by Gehring et al. (74) who used an electron capture detector, instead of the flame-ionization detector used in their study.

The National Human Monitoring Program for Pesticides, through its cooperative arrangement with the Health and Nutritional Examination Survey II (Hanes II project), is currently analyzing human urine samples for silvex, 2,4,5-T, and 2,4,5-TCP (64). The survey is scheduled for completion in 1979, but some extremely tentative results are available. No quantifiable 2,4,5-T residues have been detected in the first 400 samples; however, trace amounts (<10 ppb) have been found in a few samples.

Dougherty and Piotrowska (177) reported on screening of human urine for environmental contamination with toxic

residues by negative chemical ionization mass spectrometry. The procedure is based on solvent extraction with minimal clean-up followed by examination with negative chemical ionization mass spectrometry for organochlorine residues and related compounds with masses greater than 130 daltons. Urine for the screening procedure was obtained from students at Florida State University (25 dorm residents; 21 football team members; and 11 swimming team members). The authors reported that the limited survey of human urines indicates contamination of the subjects with 2,4,5-T, pentachlorophenol, other polychlorophenoxy acids, and numerous unknown compounds. The authors indicated that 2,4,5-T was found in 36% (9/25) of the dorm residents; 24% (5/21) of the football team; and 9% (1/11) of the swimming team. The authors attempted to define the source of the contamination by applying the same screening procedure to environmental substrates and suggested the food chain (beef fat in the case of 2,4,5-T) as one significant source of the contamination.

(7) Animal Products

Kocher et al. (76) surveyed beef fat from cattle grazing on land where 2,4,5-T had been applied to determine if TCDD was present in this tissue. None of the 2,4,5-T samples used were available for analysis for TCDD content.

The authors did not know whether the samples were produced before 1972 (when maximum allowable TCDD content was 1 ppm) or after 1972 (when maximum allowable TCDD content was 0.1 ppm). None of the 16 samples from Sugarland, Texas, Missouri, and Oklahoma showed TCDD residues when analyzed by a gas chromatography-mass spectrometry detection technique (detection limits: 3 to 6 ppt). Three of the eight samples from Mertzon, Texas, where animals had grazed for 30 days in a fenced pasture sprayed in its entirety with 2,4,5-T, gave positive responses at the detection limit of 3 to 4 ppt TCDD.

In another surveillance study, Mahle et al. (77) analyzed milk from cows grazing on grass treated with 2,4,5-T in accord with normal agricultural practices. Twenty-five samples were collected from different farms in Oklahoma, Arkansas, and Missouri; these areas were selected as representative of those where 2,4,5-T is used to control broadleaf weeds and brush in pasture and rangeland. Milk purchased in Midland, Michigan, an area where 2,4,5-T is not used, provided control samples. Based on gas chromatography-mass spectrometry data (detection limit: 1 ppt), the authors stated that control samples were indistinguishable from the samples from treated areas and concluded that TCDD was not present.

The residue levels reported in animal products in the studies cited below were obtained in laboratory feeding studies and not from animals grazing on pastures and rangelands treated at dosage rates recommended on registered product labels. Nevertheless, residues obtained in these feeding studies could occur in the environment and at these same levels since animals grazing on forage plants immediately after treatment at recommended rates of application could ingest 2,4,5-T in amounts similar to those fed in the studies.

Leng (37) found no residues greater than 0.05 ppm in milk or cream of cows ingesting 10 to 30 ppm 2,4,5-T. At 100 ppm 2,4,5-T in the diet, traces of 2,4,5-TCP (0.06 ppm) were found in milk and cream. When the diet contained high

levels of 2,4,5-T, equivalent to 300 and 1,000 ppm in the total diet, residues of 2,4,5-T and 2,4,5-TCP ranged from 0.15 to 0.5 ppm in milk of individual cows.

Leng (37), reporting on residues in meat and meat byproducts, stated that calves slaughtered after ingesting 300 ppm 2,4,5-T in the total diet contained average residues of 0.12 to 0.28 ppm in muscle, fat, and liver, and 3.3 ppm in kidney. Animals fed 900 to 2,000 ppm 2,4,5-T in the total diet and slaughtered without withdrawal had proportionally higher average residues in tissue. No residues were detected (detection limit: 0.05 ppm) in most tissues when animals were given untreated feed for one week after they had been on the highest levels (1,800 and 2,000 ppm) of 2,4,5-T for four weeks. Residues of 2,4,5-T declined rapidly in tissues as soon as animals started to eat untreated feed.

Clark and Palmer (78) found 0.08 ppm 2,4,5-T in omental fat of each of two sheep given four oral doses of either 0.15 or 0.75 mg/kg of the propylene glycol butyl ester of 2,4,5-T. They also found 368 ppm 2,4,5-T in kidneys of animals killed by four daily 250 mg/kg doses of a 2,4,5-T ester.

Clark et al. (36) found 2,4,5-T no higher than 0.05 ppm in muscle or fat of sheep held one week on untreated

feed. Residues of the metabolite 2,4,5-TCP were not detected in the fat of any of the animals. They also found that the 2,4,5-T level in liver and kidneys was less than 0.05 ppm after the animals were on untreated feed for seven days.

Leng (79) found low levels of 2,4,5-T in muscle and fat of calves receiving 300 to 900 ppm 2,4,5-T in the diet and much higher residues in tissues of animals fed 1,800 ppm 2,4,5-T for 28 days. Calves fed 300 ppm 2,4,5-T showed 0.12 and 0.28 ppm 2,4,5-T in muscle and fat, respectively. Calves fed 900 ppm showed 0.24 and 0.38 ppm in muscle and fat, respectively. And at 1,800 ppm in the diet, calves showed 1.2 and 2.0 ppm in muscle and fat, respectively. In this same study, Leng found relatively low residues of 2,4,5-T in liver at feeding levels of 300 and 900 ppm (0.2 and 1.0 ppm, respectively) and sharply increased residues (7.9 ppm) at 1,800 ppm, indicating that the threshold level may have been exceeded at this higher dosage level. Residues of 2,4,5-T in kidney appeared to be proportional to the level in the diet.

Eighty-five samples of beef fat were analyzed for TCDD content under the auspices of the EPA Dioxin Implementation Plan (see Section II). These beef fat

samples included 18 samples from control areas and 67 samples from areas previously treated with 2,4,5-T. None of the 18 control samples had detectable amounts of TCDD at a detection limit of 10 ppt. Of the 67 samples from areas previously exposed to 2,4,5-T, one showed a positive TCDD level of 60 ppt; two appeared to have TCDD at 20 ppt; and five may have had TCDD levels which ranged from 5 to 10 ppt. The values for these five samples were at or below the limits of detection of 10 ppt. Forty-three beef liver samples were analyzed and showed no TCDD residues at a detection limit of 10 ppt.

(8) Food

Evidence that very little 2,4,5-T gets into food is seen in results of Market Basket Surveys conducted by the Food and Drug Administration (FDA). Of the 134 total diet samples involving 1,600 food composites (Market Basket Survey) analyzed from 1964 through April 1969, only three contained 2,4,5-T. Two were dairy products containing 8 to 13% fat with 0.008 and 0.19 ppm in the fat. A single meat, fish, and poultry composite from Boston consisting of 17 to 23% fat was found to contain 0.003 ppm 2,4,5-T on a fat basis (81, 82, 83, 84).

FDA Market Basket Survey samples from 1969 through July 1974 showed no 2,4,5-T residues (detection limit: 0.02

ppm) in 155 total diet samples involving 1,869 food composites (85, 86, 87, 88, 89).

(9) Human Exposure via Industrial Accidents

There have been a number of industrial accidents during manufacture of chlorinated phenols that have resulted in human exposure to TCDD.

Whiteside (90) reported on a 1949 explosion at a chemical plant producing 2,4,5-T in Nitro, West Virginia. The release of intermediate chemicals led to 228 cases of chloracne among exposed workers. Whiteside stated that symptoms of affected workers included skin eruptions, shortness of breath, intolerance to cold, palpable and tender liver, loss of sensation in extremities, damage to peripheral nerves, fatigue, nervousness, irritability, insomnia, loss of libido, and vertigo.

Goldmann (91) reported on a 1953 accident at a 2,4,5-TCP production plant in Germany. Temperature and pressure rose explosively in the autoclave, forming previously unknown, very toxic chlorinated hydrocarbons; 42 persons contracted serious cases of dermatitis, in which 14 persons suffered consequent damage to internal organs, and seven persons experienced disturbances of the nervous system.

A similar accident occurred in Amsterdam in 1963 when an explosion in a 2,4,5-T factory resulted in 50 workers contracting chloracne (90).

In 1954, 31 workers in a Hamburg, Germany, chemical plant producing 2,4,5-T from technical 2,4,5-TCP contracted chloracne (10, 16, 92) and suffered the physical and psychological symptoms associated with it (93). Kimmig and Schulz (10) extensively investigated the workers' conditions and conducted experiments treating the skin of a rabbit's ear with chemicals to which workers had been exposed. These researchers tentatively identified the causative agent of the chloracne as TCDD. Bauer et al. (15) conclusively identified TCDD as the cause of chloracne.

In 1964 workers in a 2,4-D and 2,4,5-T plant in the United States developed chloracne (93, 94). Bleiberg et al. (94) found evidence of porphyria cutanea tarda (PCT) of varying degrees of severity in 11 out of 29 workers. PCT had never before been described as related to chloracne, nor had it been ascribed to industrial exposure in the United States. The authors stated that either the finished chemicals or some intermediate were responsible for both diseases.

The Fine Chemicals Unit of Coalite and Chemical Products Limited located at Bolsover, Derbyshire, in England had been producing 2,4,5-TCP for nearly three years without

incident when an explosion occurred at midnight on April 23, 1968. As a result of this exothermal reaction, TCDD had accidentally been produced. Workers at this plant were accidentally exposed to TCDD, and 79 cases of chloracne were recorded, many of them severe (9, 95).

Beginning in May 1971 an accidental poisoning episode occurred in the United States that affected humans, horses, and other animals. The exposure was related to the spraying of waste oil, contaminated with TCDD, on riding arenas to control dust. Three days after spraying, sparrows and other birds were found dead on the arena floor. Of 85 horses exercised within the arena, 62 became ill, and 48 died. The first horse died on June 20, 1971. Horses continued to die as late as January 1974. Human illnesses were less severe, but did include one case of hemorrhagic cystitis in a 6-year-old girl who frequently played in the arena. Analysis showed the arena contained 31.8 to 33.0 ug/g TCDD (96, 97, 162).

Beale et al. (98) presented follow-up information on the 6-year-old girl involved in this accidental poisoning. These authors stated that the girl's symptoms resolved in three to four days and did not recur. Results of a repeat voiding cystogram three months later appeared normal. Cystoscopy at this time did, however, demonstrate numerous punctate haemorrhagic areas in the bladder, especially in

the region of the trigone. Five years later, an investigation showed that this girl had grown normally; results of a physical examination, including a detailed neurological examination, were normal. Cystogram and liver-function tests were also normal, as was the urinary excretion of uroporphyrins, coproporphyrins, and thyroid function.

On July 10, 1976, an accident at the ICMESA chemical plant in the Seveso Region of Italy released 2 to 10 pounds of TCDD over a wide area (90, 99, 100). Hundreds of animals died, many area residents reported skin disorders, and an area of 110 hectares was evacuated (101). Reports of the immediate symptoms and indications of many long-term effects are just becoming available.

Seveso inhabitants initially experienced numerous, burnlike skin lesions which gradually receded; Whiteside (90) believed this type of lesion was probably due to direct contact with the sodium hydroxide and phenolic components of the fallout. Two and a half months after the explosion, however, children and young people in the zone most affected by the fallout developed symptoms of true chloracne, a sign of dioxin poisoning, on their faces, arms, and bodies. By November 1976, 28 people had developed confirmed cases of chloracne, and the number rose to 38 by December 1976; one year later, the number of confirmed cases of chloracne was 130.

A number of Seveso women were pregnant at the time of the accident. Whiteside (90) reported that the number of legal and illegal abortions performed after the accident probably totalled 90. Results of a survey by an epidemological commission showed that 183 babies were delivered in the two months following the accident and that there were 51 spontaneous abortions as distinct from induced abortions (approximately double the rate of spontaneous abortions previously reported for the area). Whiteside (90) reported that eight cases of birth abnormalities have been noted to date among babies born to women in the Seveso area who were pregnant at the time of the explosion. Physicians in the Seveso area have had difficulty relating this directly to the explosion, however, since this incidence of birth abnormalities was not disproportionate to the usual incidence of abnormal births.

H. Tolerances

There are no tolerances established for 2,4,5-T in or on food crops. Likewise, no tolerances have been set specifically for TCDD in or on food crops. However, 40 CFR Section 180.302 does establish a tolerance of 0.05 ppm for hexachlorophene on cotton seed (a nonhuman dietary food item), with a stated limitation that the technical grade

hexachlorophene used in the formulation shall not contain more than 0.1 ppm TCDD. The limitation does not constitute a tolerance (102).

I. Pesticide Episods Reports System (PERS)

EPA's Pesticide Episode Response Branch of the Office of Pesticide Programs maintains a Pesticide Episode Reports System (PERS) which collects reports of pesticide exposure affecting humans, domestic animals, livestock, and wildlife (103). According to their records, there were 96 episodes from 1966 to April 1977 involving 2,4,5-T.

Many of these 96 episodes recorded effects in more than one area of the environment. Plant damage was reported 60 times, effects on humans 16 times, water contamination 14 times, effects on domestic animals and soil contamination 7 times each, general environmental contamination 3 times, and fish kills and complaints against use of 2,4,5-T twice each.

There was substantial evidence in 13 of the 96 episodes linking 2,4,5-T to the episode's effects; there was circumstantial evidence in 20 of the episodes for involvement of 2,4,5-T; there was insufficient evidence in 62 of the episodes to prove or disprove involvement of 2,4,5-T; and one episode had no verification status listed.

Of the 13 episodes for which there was substantial evidence linking 2,4,5-T to the episode's effects, two involved humans (including one suicide); 2,4-D was also involved in both episodes. Three episodes involved plant damage from drift of herbicides; 2,4,5-T residues were found in plant samples in two episodes; 2,4-D was also involved in one of these episodes. Two episodes involved fish kills resulting from accidental spills into streams, with 2,4-D involved in both incidents; in one of these episodes, 6,000 fish (90% juvenile salmon) were killed; residues of both 2,4-D and 2,4,5-T were found in these fish. Two incidents involved soil contamination when two warehouses were destroyed by a tornado and fire; many other pesticides were involved in both instances. Two episodes involved domestic animals; in one, 24 cows died after herbicide application. Arsenic residues were found in two cows, and arsenic contamination of the herbicide mix was suspected. In the other instance, 8 cows drank water contaminated with 2,4,5-T; residue levels of 0.03 and 0.02 ppm were found in the milk five and eight days, respectively, after the incident. Two hundred and forty gallons of milk were dumped. One incident involved water contamination as a result of a warehouse fire; many other pesticides were also involved.

II. REGULATORY HISTORY

2,4,5-T was developed during World War II and was first registered as a pesticide on March 2, 1948 (3). Since then, it has been the subject of several Federal regulatory actions.

On April 13, 1966, the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) published an announcement in the Federal Register abolishing the "No Residue and Zero Tolerance" concepts as scientifically untenable. Future registrations would be granted on the basis of either "Negligible Residue" or "Permissible Residue." Industry was given until December 31, 1967, to comply by obtaining tolerances for residues of 2,4,5-T in all treated food, feed products, and byproducts (in addition no registrations would be continued beyond December 31, 1970).

Following this action, a series of Pesticide Registration (PR) Notices were issued over several years, extending certain "no residue" and "zero tolerance" registrations beyond the December 31, 1967, deadline for obtaining residue tolerance. (These and all following PR Notices are cited in Reference 104.) Among uses of 2,4,5-T extended beyond the deadline were uses on pasture grasses and rangeland; on apples (McIntosh), blueberries (low bush), cereal grains (undesignated), rice, and sugarcane; and in lakes and ponds.

PR Notice 70-8 issued by the USDA on March 10, 1970, identified data needs for certain compounds. 2,4,5-T was identified as one of the compounds requiring further teratogenic studies.

PR Notice 70-11 published on April 20, 1970, suspended 2,4,5-T products bearing certain directions for use. The suspended uses were all uses in lakes, ponds, or on ditch banks; and liquid formulations for use around the home, recreation areas, and similar sites.

PR Notice 70-13 issued by the USDA on May 1, 1970, cancelled 2,4,5-T products bearing certain directions for use. The cancelled uses were all granular 2,4,5-T formulations for use around the home, recreational areas, and similar sites; and all 2,4,5-T uses on food crops intended for human consumption.

All registrants were advised of these actions, and two of the 2,4,5-T registrants, Dow Chemical and Hercules Incorporated, excercised their right under Section 4(e) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) [7 U.S.C. 135 et seq.] to petition for referral of the cancellation (rice use only) to an Advisory Committee.

As provided by Section 4(c) of FIFRA (1964 amendment), a nine-member Advisory Committee of scientists was appointed

to consider all relevant facts, submit a report and recommendations regarding registration for certain uses of 2,4,5-T, and state the reasons or bases for these recommendations. Their report was submitted to the Administrator of the Environmental Protection Agency on May 7, 1971 (48). The Committee recommended that use of 2,4,5-T be permitted in forestry, range land, and rights-of-way providing that the limit of 0.1 ppm of contamination with TCDD be set for all future production of 2,4,5-T; that 2,4,5-T be applied no more than once a year at any one site; and that 2,4,5-T be applied with proper caution so that it will not contaminate other areas where it may come into contact with humans.

The Committee also recommended that this action be reviewed again when existing deficiencies in information about possible magnification of TCDD in the food chain have been rectified by specific research.

In the meantime, PR Notice 70-22, published by the USDA on September 28, 1970, addressed the presence of chlorodioxin contaminants in economic poisons. This notice stated that the USDA had determined that certain toxic chlorodioxins (such as TCDD) may be present as contaminants in the basic materials used in formulating 2,4,5-T and silvex. The notice also stated that the presence of such

chlorodioxins constituted a possible hazard to man since they had been found to be extremely toxic to laboratory animals, and that appropriate regulatory action would be taken under provisions of FIFRA since products containing chlorodioxins are considered to be in violation of FIFRA.

Dow Chemical obtained an injunction against EPA in July 1972, enjoining further administrative action against 2,4,5-T. The United States Court of Appeals for the Eighth Circuit overturned the injunction in 1973, and administrative proceedings were allowed to go forward.

On July 20, 1973, a notice of intent to hold public hearings on all uses of 2,4,5-T was filed with the EPA Hearing Clerk under Section 6(b)(2) of FIFRA, as amended 1972. All federally approved uses of 2,4,5-T were to be explored in a public hearing scheduled for April 1974, following completion of an intensive monitoring program for detecting dioxin in the ppt range (38 FR 19869, July 29, 1973).

On May 10, 1974, the information hearing was expanded to include all insecticides and herbicides having 2,4,5-TCP in their manufacturing process. These included silvex, erbon, and ronnel, as well as 2,4,5-T and 2,4,5-TCP, all of which may contain TCDD.

On June 24, 1974, EPA withdrew cancellation and information-gathering proceedings initiated against 2,4,5-T and related compounds because of its inability to monitor food for TCDD residues with the necessary analytical precision. Although the 2,4,5-T notice of hearing was withdrawn, the Agency stated that it "will continue its TCDD residue monitoring program and will take such further action as it deems appropriate once the results of the monitoring project are available" (39 FR 24050 June 28, 1974).

On July 25-26, 1974, the Agency held a Dioxin Planning Conference in Washington, D.C., primarily for those parties having an interest in the withdrawn 2,4,5-T/dioxin hearings, to address data analysis and retrieval (in the areas of analytical methodolgy, toxicology, and monitoring) with emphasis on analytical methodology for TCDD at the ppt level. As a result, the Agency established a Dioxin Implementation Plan (DIP) intended to identify a preferable analytical methodology to monitor human and environmental samples for TCDD.

On-going TCDD studies under the DIP include: an analytical method validation study to produce statistically defensible data; monitoring for residues in human milk in the Pacific northwest; additional beef fat residue studies;

additional technical pesticide residue studies; and an environmental monitoring program for TCDD residues in soil, water, and biota.

III. SUMMARY OF SCIENTIFIC EVIDENCE RELATING TO REBUTTABLE PRESUMPTION

The following adverse effects of 2,4,5-T and/or TCDD have been found to exceed the criteria for issuance of a rebuttable presumption as stated in Section 162.11 of the Code of Federal Regulations (CFR 40). Because of industry's apparent inability to produce 2,4,5-T without TCDD contamination, none of the studies cited are for pure 2,4,5-T. The effects of TCDD must also be considered when assessing 2,4,5-T by the Agency's risk criteria.

A. Oncogenic Effects

40 CFR Section 162.11(a)(3)(ii)(A) provides that a rebuttable presumption shall arise "if a pesticide's ingredient(s)...(i)nduces oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation or dermal exposure...." Section 162.3(bb) defines the term oncogenic as "the property of a substance or a mixture of substances to produce or induce benign or malignant tumor formation in living animals."

The studies summarized below indicate that 2,4,5-T containing less than 0.05 ppm TCDD and/or TCDD alone have oncogenic effects in two mouse strains and one rat strain. Since 2,4,5-T, as currently formulated, contains TCDD (at a maximum amount of 0.099 ppm), a rebuttable presumption against the registration of 2,4,5-T products has arisen because of the oncogenic effect of 2,4,5-T and its contaminant, TCDD.

(1) 2,4,5-T

(a) Effects of Dietary 2,4,5-T (<0.05 ppm TCDD) on Rodents

In their bioassy of 2,4,5-T for carcinogenicity in mice, Muranyi-Kovacs et al. (105) administered 2,4,5-T (containing <0.05 ppm TCDD)[6/] to inbred C3Hf and XVII/G mice. The mice were given 100 mg/liter of 2,4,5-T in the drinking water for two months beginning at six weeks of age. During the succeeding 15 to 20 months, the mice were given 2,4,5-T mixed in the diet at a concentration of 80 ppm ad libitum.

In C3Hf mice, 48% of the treated females (12/25) and 55% of the treated males (12/22) developed tumors, compared with control values of 21% (9/44) and 49% (21/43), respectively (Table 2). The differences between the number of tumors observed and the number expected were significant for female mice at all sites ($p < 0.03$) and for the combined sexes

6/ This TCDD level is less than the 0.1 ppm TCDD currently found in most commercial formulations (see Section I.B).

($p < 0.01$).[I/] For non-incidental tumors, the differences were significant for each sex and the combination; no significant differences were found in incidental tumors.[8/] No other strain-sex combination yielded statistically significant values (106). Rare types of tumors, not seen in the control animals, were observed in the treated C3Hf females.

Table 2. Oncogenic Effects of 2,4,5-T on Mice[a/]

Strain	Sex	Dietary Level (ppm)[b/]	Mean Survival Time (days)	Mice with Leukemia and Lung and Liver Tumors No/Total No[c/]	%	Incidence of Tumors: Total	Lung	Liver	Leukemia	Other
C3HF	M	0	630	21/43	49	22	2	19	--	1[d/]
		80	511[k/]	12/22	55	13	--	10	2	1[e/]
	F	0	680	9/44	21	9	5	3	1	--
		80	620	12/25	48	13	--	4	3	6[f/]
XVII/G	M	0	521	25/32	78	27	22	4	--	1[g/]
		80	583	15/20	75	16	14	--	1	1[h/]
	F	0	569	21/40	53	24	20	--	2	2[i/]
		80	641[l/]	16/19	84	16	15	--	1	--

a/ Data from Muranyi-Kovacs (105).
b/ Estimated daily oral dose = 12 mg/kg body weight.
c/ Effective number of mice are mice surviving longer than 300 days or developing a tumor before 300 days of age.
d/ Pleomorphic salivary gland tumor.
e/ Fibrosarcoma; one hyperplastic urinary bladder and one hyperplastic forestomach not included.
f/ One osteogenic sarcoma; two sarcomas; two cutaneous tumors; one cervical tumor.
g/ Forestomach tumor.
h/ Urinary bladder papilloma; two hyperplastic lesions of urinary bladder not included.
i/ Two hemangiomas.
k/ $p < 0.01$ compared with controls.
l/ $p < 0.001$ compared with controls.

I/ The investigators found no significant sex-related differences.

8/ Incidental tumors are tumors discovered at necropsy of an animal which died from some other cancer; non-incidental tumors are tumors diagnosed during life or which caused the death of the animal.

A decrease in survival time for mice with tumors was noted in both male and female treated C3Hf mice when compared with controls. C3Hf treated male mice survived an average of 511 days compared with 630 days for control male mice. According to the evaluation by EPA's Carcinogen Assessment Group [CAG] (106), this difference was significant ($p < 0.001$). Treated female C3Hf mice survived 620 days compared with 680 days for control females. Chemically induced oncogenic effects typically show long latency periods. The finding of reduced longevity among treated animals as compared with controls complicates the assessment of the potential oncogenic effects of 2,4,5-T.

In XVII/G mice, 84% of the treated females (16/19) and 75% of the treated males (15/20) developed tumors, compared with control values of 53% (21/40) and 78% (25/32), respectively.

An increase in survival time for mice with tumors over controls was noted among the XVII/G treated animals. There was an average survival time of 583 days for treated male mice compared with 521 days for control male mice. Treated females survived 641 days compared with 569 days for control females. According to CAG (106), the difference was significant ($p < 0.01$) in females.

(b) Effects of Subcutaneous Injection and Oral Administration of 2,4,5-T (30 ppm TCDD) on Rodents

Innes et al. (107) studied the tumorigenicity of 2,4,5-T, containing about 30 ppm TCDD, in two hybrid strains of mice, designated as "X" and "Y", after oral or subcutaneous administration of the maximum tolerated dose (Table 3). The testing was performed at Bionetics Research Laboratories, under contract from the National Institutes of Health. Results of the studies were calculated comparing treated groups with matched and pooled controls.9/

In the subcutaneous study, mice were given a single injection of 21.5 mg/kg of 2,4,5-T in a dimethyl sulfoxide (DMSO) solution at approximately 18 months of age. Seventeen percent (3/18) of the treated "Y" males developed pulmonary adenomas, compared with 1% (1/71) of the matched controls and 3% (4/122) of the pooled controls. This increased incidence of pulmonary adenomas was significant relative to both control groups [p = 0.024 matched and p = 0.04 pooled] (106).

In the oral study, 21.5 mg/kg of 2,4,5-T in gelatin was administered daily by stomach tube, beginning at seven

9/ Because this was a large scale screening study, several control groups were used. No significant differences were found among these groups.

days of age. After weaning, 60 ppm of 2,4,5-T was mixed in the diet and provided ad libitum until the end of the study at approximately 18 months. Gross and histological examinations were made of all major organs and visible lesions; thyroid glands were not examined. According to CAG's evaluation (106), there were no significant differences between 2,4,5-T treated and control groups of mice with respect to tumors at specific sites or total number of tumor-bearing animals.

Table 3. Tumors in Mice Ingesting 2,4,5-T

Strain	Sex	Dose (ppm)	Mice with Tumors		Mice with Specific Tumors		
			No/Total No	%	Reticulum Cell Sarcoma	Tumor Type Pumonary Adenoma & Carcinoma	Hepatoma
X	M	0 (matched)	5/15	33	0	2	3
		0 (pooled)	22/79	28	5	5	8
		60	6/18	33	1	1	4
	F	0 (matched)	2/18	11	1	1	--
		0 (pooled)	8/87	9	4	3	--
		60	1/81	6	--	1	--
Y	M	0 (matched)	3/18	17	--	3	--
		0 (pooled)	16/90	18	1	10	5
		60	3/18	17	2	--	1
	F	0 (matched)	1/15	7	1	--	--
		0 (pooled)	7/82	9	3	3	1
		60	2/18	11	1	--	--

(2) TCDD

(a) Oncogenic Effects of Low Levels of TCDD on Rodents

Van Miller et al. (109) recently reported the results of a two-year feeding study with male Sprague-Dawley rats. Ten groups of ten animals per group were fed ground chow containing 0, 1, 5, 50, or 500 ppt (= 10^{-12} gram TCDD/gram food), and 1, 5, 50, 500, or 1,000 ppb (= 10^{-9} gram TCDD/gram food) TCDD.

Food intake (10 ± 4 g/day) was significantly lower in rats ingesting the three highest dose levels (50, 500, or 1,000 ppb TCDD) than in controls (21 ± 2 g/day), and none of the rats in these three groups gained weight after the start of the experimental diet. All rats receiving these three dose levels died between the second and fourth week of treatment.

On the other hand, food intake for rats on other dose levels was similar to controls (20 ± 2 g/day). Weight gain was significantly less only for rats given 5 ppb TCDD (391 ± 54 g) as compared to controls (531 ± 44 g). In these

seven groups only one animal died before the 30th week, and that death occurred in the 500-ppt group at the 17th week. In the 5- and 1-ppb groups, all animals died by the 90th week of the experiment. Table 4 shows the mortality figures for all groups.

Table 4. Mortality in Rats Ingesting Various Levels of TCDD

Dose	Week of First Death	No. Rats Dead at 95th Week[a/]
0[b/]	68	6/10 (60%)
1 ppt[c/]	86	2/10 (20%)
5 ppt[d/]	33	4/10 (40%)
50 ppt[e/]	69	4/10 (40%)
500 ppt[f/]	17	5/10 (50%)
1 ppb[g/]	31	10/10 (100%)
5 ppb[h/]	31	10/10 (100%)
50 ppb[i/]	3	10/10 (100%)
500 ppb[j/]	2	10/10 (100%)
1,000 ppb[k/]	2	10/10 (100%)

a/ Surviving animals sacrificed at 95 weeks.
b/ Control group. Diet contained no TCDD.
c/ Approximate weekly dose was 0.0003 ug/kg body wt.
d/ Approximate weekly dose was 0.001 ug/kg body wt.
e/ Approximate weekly dose was 0.01 ug/kg body wt.
f/ Approximate weekly dose was 0.1 ug/kg body wt.
g/ Approximate weekly dose was 0.4 ug/kg body wt.
h/ Approximate weekly dose was 2.0 ug/kg body wt.
i/ Approximate weekly dose was 24 ug/kg body wt.
j/ Approximate weekly dose was 240 ug/kg body wt.
k/ Approximate weekly dose was 500 ug/kg body wt.

Laparotomies were performed on all rats surviving through the 65th week, and all tumors observed were biopsied. Rats were maintained on these diets until the 78th week and were then placed on the control diet. Surviving animals were killed at 95 weeks. Complete necropsies were done at death or sacrifice, and tissue samples were microscopically examined. Special staining methods were used to "aid in the diagnosis of neoplasms."

Tumorigenic and toxic effects were observed in rats in the six lowest dose groups. The overall incidence of neoplasms in the six experimental groups was 38% (23/60), compared with 0% (0/10) in the control group. The difference is statistically significant (106). Neoplastic nodules and cholangiocarcinomas of the liver were observed in 40% (4/10) of the rats ingesting 5 ppb TCDD; two animals had both neoplastic nodules of the liver and cholangiocarcinomas. One rat (10%) in the 1 ppb group had hepatic carcinoma compared to none of the controls. Hepatic tumors were not found in other dose groups (Table 5).

Table 5. Liver Tumors in Rats Ingesting TCDD[a/]

	Rats With Neoplastic Nodules		Rats With Cholangio-carcinomas		Rats With Nodules plus Carcinomas	
Dose (ppb)	No.	%	No.	%	No.	%
0	0/10	0	0/10	0	0/10	0
1	0/10	0	1/10	10	1/10	10
5	4/10	40[b/]	2/10	20[b/]	4/10	40[b/]

a/ Data from Van Miller (109).
b/ Two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

Tumors developed in 46% (23/50) of the rats ingesting 5, 50, or 500 ppt and 1 or 5 ppb TCDD, compared to none (0/10) in the control rats. Van Miller et al. noted that "nineteen (57%) [sic - Agency calculation is 54% (19/35)] of the animals that died in the six groups fed subacute levels of TCDD had neoplastic alterations." Carcinomas were observed in the ear duct, kidney, and liver. Three retriperitoneal histiocytomas were described as metastasizing to the "lungs, kidney, liver, and skeletal musculature." According to CAG's evaluation (106), statistically significant increases in tumors at all sites were found in rats fed 5, 500, 1,000, and 5,000 ppt as compared with control animals ($p=0.05$) [Table 6]. Three of the ten deaths which occurred in the 5-ppb dose group were attributed to aplastic anemia. One animal in the 500-ppt group had a severe liver infarction.

Dow Chemical USA (110) has provided EPA with a preliminary report of a study of TCDD's chronic toxic effects in Sprague-Dawley rats. Groups of 50 rats of each sex were fed 0.1, 0.01, or 0.001 ug TCDD/kg body weight daily for two years. To provide these dose levels, the concentrations of TCDD in the diet were approximately 2,200, 210, and 22 ppt. Eighty-six animals of each sex were used as controls.

Dow (110) reported "discernible increases" in the incidence of hepatocellular carcinomas of the liver and of squamous cell carcinomas of the lung, hard palate/nasal

Table 6. Total Tumors in Rats Ingesting TCDD[a/]

Dose[b/]	Tumors			Rats With Tumors	
	Benign	Malignant	Total	No.	%
0	0	0	0	0/10	0%[c/]
1 ppt	0	0	0	0/10	0%
5 ppt	1	5	6[d/]	5/10	50%[e/]
50 ppt	2	1	3[f/]	3/10	30%
500 ppt	2	2	4[g/]	4/10	40%[h/]
1 ppb	0	5	5[i/]	4/10	40%
5 ppb	8	2	10[j]	7/10	70%

a/ Data from Van Miller (109).
b/ Rats administered 50, 500, and 1,000 ppb were all dead within four weeks.
c/ Forty male rats used as controls for another study that were received at the same time and kept under identical conditions did not have neoplasms when killed at 18 months.
d/ One rat had ear duct carcinoma and lymphocytic leukemia. The following tumor types were each observed in one rat: adenocarcinomas (kidney), malignant histiocytoma (retroperitoneal), angiosarcoma (skin), and Leydig cell adenoma (testis).
e/ Three rats died with aplastic anemia.
f/ The following tumor types were each observed in one rat: fibrosarcoma (muscle), squamous cell tumor (skin), and astrocytoma (brain).
g/ The following tumor types were each observed in one rat: fibroma (striated muscle), carcinoma (skin), sclerosing seminoma (testis), and adenocarcinoma (kidney).
h/ One rat had a severe liver infarction.
i/ One rat had cholangiocarcinoma and malignant histiocytomas (retriperitoneal). The following tumor types were each observed in one rat: angiosarcoma (skin), glioblastoma (brain), and malignant histiocytoma (retroperitioneal).
j/ One rat had squamous cell tumor (lung) and neoplastic nodule (liver). Two rats had cholangiocarcinoma and neoplastic nodule (liver). Three rats had squamous cell tumors (lung). One rat had neoplastic nodule.

turbinates, and tongue in rats at 0.1 ug/kg. They also reported decreased incidences of pituitary, uterine, mammary gland, pancreatic, and adrenal gland tumors at this dose level. Dow also reported that this dose level produced increased mortality, decreased body weight gain, and changes in blood chemistry values which suggested severe toxicity. Hepatocellular nodules and alveolar hyperplasia were observed in the 0.01 ug/kg group. A squamous cell carcinoma of the hard palate was observed in one female receiving this dose; Dow considered this unrelated to TCDD treatment because a similar tumor occurred in "other concurrent studies." At 0.001 ug/kg there were no "discernible effects in male rats and an increased incidence of [reversible] swollen hepatocytes in female rats."

Dow's preliminary report does not include control data, quantitative data on tumor incidence, or statistical analyses. CAG has not evaluated this study. Table 7 describes the available tumor information. Dow has submitted the final report for this study, which CAG is currently reviewing.

Table 7. Tumors in Sprague-Dawley Rats Ingesting TCDD[a/]

Dose		Tumors
ug/kg/day	ppt	
0	0	----
0.001	22	----
0.01	210	Hepatocellular Nodules
		Squamous Cell Carcinoma[b/]
		Alveolar Hyperplasia
0.1	2,220	Hepatocellular Carcinoma[c/]
		Squamous Cell Carcinoma[d/]

a/ Data from Dow Chemical USA (110), a preliminary report.
b/ Hardpalate squamous cell carcinoma observed in only one female rat.
c/ Observed only in females.
d/ Squamous cell carcinoma observed in lungs, hardpalate/-nasal turbinate, or tongue.

(b) Effects Closely Related to Oncogenicity in Test Animals

Many chemically non-reactive carcinogens are enzymatically converted to biologically active carcinogens. The enzyme aryl hydrocarbon hydroxylase (AHH) is strongly implicated in this process (112). For example, the incidence of bronchiogenic carcinomas in humans (113) and mouse sarcomas induced by 3-methyl-cholanthrene (114) have been related to the level of inducibility of AHH (99).

Kouri et al. (114) studied AHH induction in human lymphocyte cultures by TCDD. The authors stated, "TCDD itself is not a potent carcinogen in mice; however, the synergistic action of TCDD with 3-methylcholanthrene (MC) produces cancer in different strains of mice in direct

proportion to the degree of elevation of the induced hydroxylase activity and associated cytochrome p_1-450 content." Their study showed a positive correlation between basal enzyme activity and enzyme levels maximally inducible by either TCDD or MC. They also found that TCDD is about 40 to 60 times more potent than MC as an inducer of hydroxylase activity in cultured human lymphocytes. These authors further suggested that, because of the relatively high levels of TCDD in certain parts of the world, TCDD may also present considerable long-term risk because of possible synergism in chemically initiated oncogenesis, in addition to short-term risks posed by its toxic and teratogenic properties.

The implication of TCDD in AHH inducibility has also been reported by Poland and Glover (115, 116) and Poland et al. (117). In their studies on chick embryo livers, Poland and Glover (115) found that all dioxins which are potent inducers have halogens at three of the four lateral ring positions and at least one nonhalogenated carbon atom. Poland and Glover (116) compared the potency of TCDD as an inducer of hepatic AHH with that of MC, the most commonly employed inducing agent. They stated that analysis of the data by a computer program for bioassay showed that TCDD was 28,640 times as potent

as MC on a molar basis. (The 95% confidence interval of the potency ratio is 2.07 to 3.95 X 10^4.) The index of precision, λ , was 0.18. Poland et al. (117) suggested that a hepatic cytosol species which binds TCDD is the receptor for the induction of hepatic aryl hydrocarbon hydroxylase.

Allen et al. (118) conducted a study in which female rhesus monkeys were fed diets containing 500 ppt TCDD for nine months. Anemia, thrombocytopenia, and leukopenia were the most debilitating changes. The altered lymphopoiesis could be associated with immune suppression. The authors reported widespread hypertrophy, hyperplasia, and metaplasia in the epithelium of monkeys exposed to TCDD, and related this to data showing increased tumor frequency in TCDD fed rats.

(3) Preliminary Epidemiological Studies

Two epidemiolgical studies lend support to a finding of increased tumorigenicity due to 2,4,5-T exposure. The English summary of a Swedish paper by Hardell (108) stated that "there were seven cases of malignant mesenchymal tumors in [87] persons [who had been] exposed to 2,4,5-T over a

period of 10-20 years." In five of the cases, exposure had been direct and comparatively massive. The latent period of 10 to 20 years is in agreement with that assumed for chemical carcinogenesis. The statistical distribution of 7 of the 87 patients deviated from the national average with a dominance of tumors in males.

Tung (120) reported an elevated incidence of primary liver cancers among Vietnamese following the wide application of "Agent Orange" as a defoliant during the years 1961 to 1962. "Agent Orange" is composed of equal parts 2,4,5-T and 2,4-D (2,4-dichlorophenoxyacetic acid) and is contaminated with TCDD. During 1962 to 1968, 10% (791/7911) of all cancers were liver cancers, compared with 3% (159/5442) during 1955 to 1961. The latent period involved is shorter than that normally assumed for chemical carcinogenesis; the possibility of a shorter latent period for some chemicals, however, cannot be eliminated. Neither of these studies is sufficient to be the basis of any firm conclusions concerning a causal connection between 2,4,5-T and cancer. But in view of the results obtained in experimental animals, they warrant noting.

The Working Group concludes that there is sufficient evidence to indicate that 2,4,5-T, containing TCDD at levels as low as 0.05 ppm, and TCDD alone can produce oncogenic effects in mammalian species. Since 2,4,5-T, as currently formulated, contains TCDD (at a maximum amount of 0.099 ppm), a rebuttable presumption against registration of 2,4,5-T products has arisen because of the oncogenic effects of 2,4,5-T and TCDD.

B. Other Chronic or Delayed Toxic Effects

40 CFR Section 162.11(a)(3)(ii)(B) provides that "a rebuttable presumption shall arise if a pesticide's ingredient(s)...(p)roduces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety." This section reflects concern that chronic exposure to chemicals may result in injury to the reproductive system and/or the fetus and provides that a rebuttable presumption shall arise if chronic chemical exposure in test animals produces such results.

The studies summarized below show that 2,4,5-T containing 0.5 ppm or less TCCD produces teratogenic and/or fetotoxic effects in mice at 30 mg/kg, in rats at 100 mg/kg, in hamsters at 40 mg/kg, and in birds at 1 mg/kg. Other

studies show that pesticide-free TCDD is fetotoxic and/or teratogenic at doses as low as 0.125 ug TCDD/kg in rats and 0.1 ug TCDD/kg in mice. Specifically, these studies show that exposure to TCDD and/or 2,4,5-T containing TCDD during pregnancy is associated with statistically significant increases in the incidence of cleft palate, kidney anomalies, skeletal and intestinal tract anomalies, and embryonic resorption. (Maternal toxicity has also been observed in many of these studies, primarily in the form of reduced weight gain and increased liver-to-body weight ratio. Whenever it has appeared particularly relevant, details have been cited in the individual studies.)

The Working Group has concluded from these studies that 2,4,5-T containing TCDD, 2,4,5-T without detectable dioxin, and TCDD alone produce fetotoxic and teratogenic effects in mammals. The Working Group has also concluded that an ample margin of safety does not exist for the population at risk (women of child-bearing age) for dermal and inhalation exposure and for cumulative oral, dermal, and inhalation exposure to both 2,4,5-T and/or TCDD. For these reasons, the Working Group recommends issuance of a rebuttable presumption based on the fetotoxic and teratogenic effects of 2,4,5-T and/or TCDD.

(1) Pesticide-free TCDD

A Bionetics Research Institute study on 2,4,5-T provided the first indication that TCDD adversely affected mammalian development (123). In this study, detailed with later confirming studies in Section III.B.(2) below, 2,4,5-T significantly increased the frequency of cleft palate, kidney anomalies, and fetal mortality in the litters of treated dams. The 2,4,5-T used in this study contained approximately 30 ppm TCDD. Subsequent studies, detailed in this section, using pesticide-free TCDD have established that TCDD alone produces these effects, and that the TCDD contaminant may be the principal chemical determinant of the fetotoxic and teratogenic effects in mammals exposed to the pesticide 2,4,5-T.

(a) Studies in which TCDD Produced Teratogenic and/or Fetotoxic Effects in Mice

Courtney and Moore (128) studied TCDD's embryotoxic and teratogenic effects in three mouse strains (Table 8). Test animals were administered 1 or 3 ug TCDD/kg body weight subcutaneously in solutions of 100% dimethylsulfoxide (DMSO) on days 6 to 15 of gestation. DMSO was administered as the control. TCDD produced cleft palates in all three strains. At 3 ug/kg, 30% (3/10) of the CD-1 litters had fetuses with cleft palates compared to 0% (0/9) of the controls; 71% (5/7) of the C57BL/6 litters had cleft palates

at 3 ug/kg as compared to 0% (0/23) of the controls; and 22% (2/29) of the DBA/2 litters had cleft palates, as compared to 0% (0/23) of the controls. The authors also found a marked increase in the incidence of kidney anomalies in all strains. One especially sensitive strain, C57BL/6, developed kidney anomalies in 100% (7/7) of the litters as compared to 9% (2/23) in the controls. Maternal liver-to-body weight ratio was significantly increased in the inbred strains, C57BL/6 and DBA/2, but not in the randomly bred CD-1 mice. TCDD had no effect on fetal mortality, fetal weights, or maternal weights at the doses administered.

Table 8. Teratogenic Effects of TCDD in Mice and Rats[a/]

Strain	Dose	Litters Affected/Live Litters				Average Fetuses Affected/Live Litters			
	(ug/kg)	Cleft Palate		Kidney Anomalies		Cleft Palate		Kidney Anomalies	
		#	%	#	%	#	%	#	%
Mouse									
CD-1	0(DMSO)	0/9	0	3/9	33	0/9	0	1/9	11
	1	1/9	11	5/9	56	2/9	22	4.6/9	51
	3	3/10	30	10/10	100	1/10	10	6.5/10	65
DBA/2	0(DMSO)	0/23	0	3/23	13	0/23	0	1/23	4
	3	2/29	22	8/9	89	1/9	11	1.8/9	20
C57BL/6	0(DMSO)	0/23	0	2/23	9	0/23	0	1/23	4
	3	5/7	71	7/7	100	2.6/7	37	3/7	43
Rat									
CD	0(DMSO)	0/9	0	0/9	0	0/9	0	0/9	0
	0.5	0/6	0	4/6	67	0/6	0	1.8/6	30

a/ Data from Courtney and Moore (128).

In another study in which six dioxins were administered subcutaneously and orally to CD-1 mice, Courtney (133) found TCDD to be the most fetotoxic and teratogenic of the dioxin compounds, by either route of exposure at all dose levels tested (Table 9). On days 7 to 16 of gestation,

TCDD was administered orally at 25 to 400 ug/kg body weight and subcutaneously at 25 to 200 ug/kg.

Mortality per litter increased with the dose and reached 97% (oral) and 76% (subcutaneous) in the litters administered TCDD, as compared with a mortality of 6 and 14% in the oral and subcutaneous control groups, respectively. The most common anomalies observed were cleft palates and malformed kidneys. All of the fetuses in the 200 and 400 ug/kg (oral) and 200 ug/kg (subcutaneous) groups exhibited cleft palates as compared to 0% of the controls. Of the fetuses in the 200 ug/kg (oral) group, 100% had kidney malformations as compared to 1% of the controls. Other anomalies observed were hydrocephalus, open eye, and club foot. Edema and petechiae were also observed in fetuses administered the high doses.

Table 9. Fetotoxic and Teratogenic Effects of TCDD in CD-1 Mice[a/]

Dose (ug/kg per day)	Route of Administration	% Average Fetal Mortality/Litter	Average # Abnormal Fetuses per Litter	Abnormal Anomalies/Total Fetuses: Cleft Palate %	Kidney Anomalies %	Club Foot %
25	Oral	6	4.6	3	34	3
50	Oral	13	8.1	19	72	7
100	Oral	14	8.3	66	71	13
200	Oral	87	1.5	100	100	14
400	Oral	97	0.4	100	50	50
25	Subcutaneous	36	6.7	82	53	11
50	Subcutaneous	56	5.0	79	58	17
100	Subcutaenous	72	3.5	85	95	0
200	Subcutaenous	76	3.1	100	38	18
5% anisole corn oil (0.1 ml)	Oral	6	0.8	0	1	4
DMSO[b/]	Subcutaneous	14	0.2	0	0	1

a/ Data from Courtney (133).
b/ DMSO = dimethylsulfoxide.

Moore et al. (174) also found that TCDD caused fetotoxic and teratogenic responses in C57BL/6 mice at 1 ug/kg administered on days 10 through 13 of gestation. Compared with 0% incidence (0/27) in the control litters, 94% (15/16) of the treated litters exhibited kidney anomalies, and 19% (3/16) had cleft palates. At 3 ug/kg, the incidence of these anomalies was 100% (14/14) and 86% (12/14), respectively.

Neubert and Dillman (127) tested the embryotoxic and teratogenic effects of TCDD in NMRI mice (Table 10). In one test, pregnant mice were given varying doses of TCDD (0.3 to 9 ug/kg) by intubation on days 6 to 15 of gestation. At 9 ug/kg, 100% (3/3) of the viable litters had resorptions; 67% (6/9) of all litters had total resorptions. Oil control values were 32 and 0% for litters with resorptions and litters with total resorptions, respectively. Cleft palate was observed in all of the litters and 82% of the fetuses at 9 ug/kg; comparable oil control values were 6 and 0.7%, respectively. Statistically significant ($p < 0.01$) proportions of the fetuses evidenced cleft palate at 3, 4.5, and 9 ug/kg (3, 13, and 82%, respectively) when compared with the oil control.

Table 10. Embryotoxic and Teratogenic Effects of TCDD on NMRI Mice[a/]

Dose[b/] (ug/kg)	Litters Affected/Viable Litters: Resorptions #	%	Cleft Palate #	%
0	23/95	24	6/95	6
oil	21/65	32	4/65	6
0.3	7/13	54	0/13	0
3.0	16/24	67	7/24	29
4.5	5/12	42	6/12	50
9.0	3/3	100	3/3	100
9.0	3/6	50	5/6	83

a/ Data from Neubert and Dillman (127).
b/ All doses administered on days 6 to 15, except second 9.0 ug/kg dose which was administered on days 9 to 13.

In this study, a single oral dose of 45 ug/kg TCDD on day 6 produced resorption in 100% of the viable litters; 23 ug/kg on day 10 led to 50% resorptions. Seventy-one per cent of the viable litters had embryos with cleft palate when 45 ug/kg was given as a single dose on day 11. Control values were 24% for litters with resorption and 6% for litters with cleft palates.

Smith et al. (135) administered 0.001, 0.01, 0.1, 1.0, and 3.0 ug TCDD/kg body weight per day to CF-1 mice by gavage from days 6 through 15 of gestation (Table 11). The percentage of resorptions per implantation was significantly higher in treated mice than in the controls only in the 1.0 ug/kg group. Cleft palate occurred in 71% of the litters treated at 3.0 ug/kg and in 21% of the litters treated at 1.0 ug/kg; bilateral dilated renal pelvises occurred in 28%

of the litters treated at 3.0 ug/kg, and in 5% of the litters treated at 1.0 ug/kg. No significant increase in either cleft palate or dilated renal pelvis was observed at 0.1, 0.01, or 0.001 ug/kg. None (0/34) of the control litters had cleft palate or abnormal kidneys.—

Table 11. Fetotoxic and Teratogenic Effects of TCDD in CF-1 Mice[a/]

Dose (ug/kg)	Incidence of Cleft Palate in Litters per Live Litters		Litters With Resorbed Fetuses per Live Litters		Litters With Dilated Renal Pelvis per Live Litters	
	#	%	#	%	#	%
0	0/34	0	25/34	74	0/34	0
0.001	2/41	5	30/41	73	0/41	0
0.01	0/19	0	17/19	89	0/19	0
0.1	1/17	6	16/17	94	0/17	0
1.0	4/19	21[b/]	18/19	95	1/19	5
3.0	10/14	71[b/]	11/14	78	4/14	28[b/]

a/ Data from Smith et al. (135).
b/ Statistically different from controls by the Fishers exact probability test ($p < 0.05$).

Neubert et al. (175) estimated the ED-50 for cleft palate in fetuses to be 40 ug TCDD/kg per day (Table 12). The no-effect-level during days 6 to 15 of gestation was estimated to be 2 ug/kg per day for NMRI mice. No pronounced fetal mortality was observed when 3 ug TCDD/kg body weight was administered on days 6 to 15 of pregnancy.

Table 12. Occurrence of Cleft Palate in Offspring of Mice Fed TCDD[a/]

Strain	Dose (ug/kg)	% Cleft Palates per Total Fetuses Examined	Affected Litters/Total Litters	
			#	%
CD-1	0	<0.3	0/29	0
	3	3	3/10	30
DBA	0	<1	0/23	0
	3	4	2/9	22
NMRI	0	0.7	10/160	6
	3	3	7/24	29
C57B1	0	<1	0/23	0
	3	22	5/7	71

a/ Data from Neubert et al. (175).

(b) Studies in Which TCDD Produced Teratogenic and or Fetotoxic Effects in Rats

Sparschu et al. (129) administered TCDD to Sprague-Dawley rats by gavage at 0.03, 0.125, 0.5, 2.0, and 8.0 ug/kg per day on days 6 through 15 of gestation (Table 13). Intestinal hemorrhages were observed in 14% (18/127) of the fetuses at 0.125 ug/kg; 36% (36/99) at 0.5 ug/kg; and 57% (4/7) at 2.0 ug/kg; none (0/246) of the control fetuses had intestinal hemorrhages. At 8.0 ug/kg per day, all fetuses (100%) were resorbed as compared to 20% (63/309) in the controls. Fetal weights were depressed at 0.125, 0.5, and 2 ug/kg. This effect was statistically significant ($p < 0.05$) in all groups except females at 0.5 ug/kg. No adverse effects were noted in the fetuses whose mothers were fed 0.03 ug/kg. The authors concluded that TCDD induced a high level of maternal and fetal toxicity and that 0.03 ug/kg per day was the no-effect-level for fetal and embryotoxic effects in rats.

Table 13. Intestinal Hemorrhages in Offspring of Sprague-Dawley Rats Fed TCDD[a/]

Dose (ug/kg per day)	Fetuses Affected/-Fetuses Examined #	Fetuses Affected/-Fetuses Examined %	Litters Affected/-Litters Examined #	Litters Affected/-Litters Examined %
0 (control)	0/246	0	0/24	0
0.03	0/115	0	0/10	0
0.125	18/127	14	7/10	70
0.5	36/99	36	10/12	83
2.0	4/7	57	2/4	50
8.0	---	---	---	---

a/ Data from Sparschu et al. (129).

Khera and Ruddick (6) studied the perinatal effects of TCDD in Wistar rats. In one test, rats were orally administered 0.125, 0.25, 0.5, and 1.0 ug TCDD/kg per day on days 6 through 15 of gestation (Table 14). Visceral lesions were observed at 0.25 ug/kg and above; slight decreases in fetal weight were also seen. Postnatal effects of prenatal exposure to TCDD were studied by allowing offspring of treated dams to be reared by untreated dams until weaning. Reduced survival, body weight gain, and reproductive ability in the progeny were observed after maternal treatment with 0.5 and 1.0 ug/kg. No fetotoxic effects were observed at 0.125 ug/kg.

In a second experiment, rats were treated orally with 1, 2, 4, 8, and 16 ug TCDD/kg body weight per day on days 6 through 15 of gestation. TCDD treatment reduced fetal weight, and the number of live fetuses per litter, and produced visceral lesions in 50% (3/6) of the 1.0 ug/kg fetuses and 43% (3/7) of the 2.0 mg/kg fetuses, as compared to none (0/10) in the controls. The incidence of skeletal anomalies was comparable to that in the controls at all dose levels. Doses of 1 ug/kg or more produced maternal toxicity; 4 ug/kg or more produced 100% embryomortality. The authors concluded that oral treatment of pregnant Wistar rats with 0.25 ug (or more)/kg per day on days 6 to 15 of gestation adversely effected rat development.

Table 14. Teratogenic Effects of TCDD in Wistar Rats[a/]

Dose (ug/kg)	Avg. # Live Fetuses/Litter	Avg. Fetal Weight (grams)	Fetuses with Skeletal Anomalies/-Total # Examined #	%	Fetuses with Microscopic Visceral Lesions/Total # Examined #	%
Test 1						
Untreated control	10.7	4.82	5/107	5	0/13	0
Treated control	11.0	4.51	21/116	18	0/11	0
0.125	10.6	4.64	3/121	2	0/38	0
0.25	10.9	4.79	6/109	6	1/33	3
0.5	10.5	4.46	10/105	10	3/31	10
1.0	9.3	4.10	6/81	7	3/10	30
Test 2						
Untreated control	11.5	4.68	8/116	7	0/10	0
Treated control	9.8	4.77	9/89	10	0/10	0
1.0	6.5	4.17	7/80	9	3/6	50
2.0	6.0	3.31	7/57	12	3/7	43
4.0	0					
8.0	0					
16.0	0					

a/ Data from Khera and Ruddick (6); treated controls given anisole-corn oil.

Courtney and Moore (128) administered TCDD to CD rats subcutaneously in solutions of 100% DMSO on days 6 through 15 of gestation (Table 8). DMSO was administered as the control. Kidney anomalies were found in four of the six litters (67%) whose dams were administered 0.5 ug/kg as compared to 0% (0/9) in the controls. TCDD did not affect fetal mortality, fetal weight, or cleft palates in the fetuses.

Dow Chemical USA (110) conducted a three-generation reproductive study on Sprague-Dawley rats continuously fed the equivalent of 0.001, 0.01, or 0.1 ug TCDD/kg per day.

A preliminary report cites reduced fertility and litter survival in f_0 rats as the reasons for discontinuing the 0.1 ug/kg dose level; significantly reduced fertility was also observed at 0.01 ug/kg. "Clearly evident" indications of toxicity at 0.01 ug/kg among f_2 and f_3 litters included smaller litter size at birth, plus decreased survival and growth of neonates. Dilated renal pelvis was observed in each of the three f_1 rats at 0.1 ug/kg which survived to adulthood. Increased frequency of this anomaly was also seen among weanlings at lower doses; however a dose-related or generational correlation could not be made. In summary, Dow concluded that "the reproductive capacity of rats ingesting TCDD was clearly affected at dose levels of 0.01 and 0.1 ug/kg per day, but not at 0.001 ug/kg per day , through three successive generations." The preliminary report did not include the numerical data necessary for Agency evaluation. Analysis will continue as these become available.

Adverse reproductive effects due to TCDD have also been observed in hamsters and chickens. Gastrointestinal hemorrhage was noted in hamster fetuses after administration of TCDD at 0.5 ug/kg per day on days 6 to 10 of gestation (48; 62). Buu Hoi et al. (111) established that 0.02 ug/kg TCDD caused teratogenic effects in chick embryos. Bowes et al. (137) and Verrett (136) confirmed these results. They found abnormalities in the beaks, eyes, and feet of chick embryos after TCDD exposure.

(c) Summary

Studies have established that TCDD is fetotoxic and teratogenic at doses as low as 0.125 ug/kg in rats (129) and at 0.3 ug/kg in mice (127); preliminary data from Dow (110) indicates that TCDD may have effects at 0.01 ug/kg in rats. Cleft palate and kidney anomalies have been observed in rats, mice, and hamsters. No fetotoxic or teratogenic effects have been observed at doses of 0.03 ug/kg in rats (129) and 0.1 ug/kg in mice (135). Table 15 lists the no-effect-levels in rats and mice for teratogenicity from TCDD.

Table 15. No-Effect-Levels for Teratogenesis from TCDD

Species	Route of Administration	No-Effect-Level ug/kg per day	Reference
Rat	Subcutaneous	<0.5	Courtney and Moore (128)
	Oral	0.125	Khera and Ruddick (6)
	Oral	0.03	Sparschu et al. (129)
Mouse	Subcutaneous	<1.0	Courtney and Moore (128)
	Oral	<0.3	Neubert and Dillman (127)
	Oral	0.1	Smith et al. (135)

(2) 2,4,5-T (TCDD Contamination Ranging From Undetectable to 30 ppm)

(a) Teratogenic and Fetotoxic Effects in Rodents

Courtney et al. (123) developed the first evidence that a 2,4,5-T pesticide product was teratogenic and fetotoxic (Table 16).[10/] The 2,4,5-T used in this study contained approximately 30 ppm TCDD. The pesticide was administered daily either orally or subcutaneously on days 6 to 14 of gestation in C57BL/6 mice, days 6 to 15 in AKR mice, and days 10 to 15 in Sprague-Dawley rats. Subcutaneous administration of 113 mg/kg body weight resulted in significant increases in the incidence of cleft palate and cystic kidneys[11/] in the embryos of both strains of mice, and fetal mortality in the C57BL/6 mice. Oral administration of

10/ Results of this study were published by the Department of Health, Education, and Welfare (121) and by Clegg (122).

11/ In a recent report on studies measuring renal alkaline phosphatase in fetal mice, Highman et al. (45) attributed the increased incidence of "cystic kidneys" in the offspring of 2,4,5-T treated animals to retarded development, rather than true teratogenesis. Reduction in fetal weight and increased incidence of cleft palate were also observed among the fetuses of treated dams.

Table 16. Teratogenic Evaluation of 2,4,5-T[a] in Mice[b]

Mouse Strain	Route of Administration	Dose (mg/kg)	# Litters	Per Litter: Avg. # Live Fetuses	Per Litter: Abnormal Fetuses %	Per Litter: % Fetuses with Cleft Palate	Per Litter: % Fetuses with Cystic Kidney	Fetal Mortality %	Abnormal Litters %
C57BL/6[c]									
Nontreated	----	---	72	5.8	11	<1	1	26	38
Control	Subcutaneous	[f]	106	5.5	12	<1	2	29	42
Control	Stomach tube	[g]	32	7.1	14	0	1	15	41
Treated	Subcutaneous	21.5[h]	6	7.7	12	0	0	3	50
Treated	Subcutaneous	113.0[h]	18	4.4	57[i]	22[i]	41[i]	42	86[i]
Treated	Stomach tube	46.4[i]	6	8.5	37[k]	2	33[i]	8	100[k]
Treated	Stomach tube	113.0[i]	12	4.8	70[i]	23[i]	48[i]	47[i]	100[i]
C57BL/6[d]									
Nontreated	----	---	8	5.1	31	0	7	36	71
Control	Subcutaneous	[f]	10	6.1	8	0	0	23	30
Treated	Subcutaneous	113.0[h]	10	7.7	77[i]	29[i]	60[i]	11	100[i]
AKR[e]									
Nontreated	----	---	58	7.1	5	<1	<1	16	19
Control	Subcutaneous	[f]	72	6.9	4	<1	<1	15	24
Control	Stomach tube	[g]	12	8.8	0	0	0	9	0
Treated	Subcutaneous	113.0[h]	14	6.9	29[i]	28[i]	1	23	71[i]
Treated	Stomach tube	113.0[i]	7	5.3	55[i]	55[i]	0	42[k]	100[i]

[a] Contained approximately 30 ppm TCDD.
[b] Data from Courtney et al. (123).
[c] Treated from day 6 through 14 of pregnancy. Killed on day 18 of gestation.
[d] Treated from day 9 through 17 of pregnancy. Killed on day 18 of gestation.
[e] Treated from day 6 through 15 of pregnancy. Killed on day 19 of gestation.
[f] Dose, 100 ul DMSO per mouse.
[g] Dose, 100 ul honey solution (honey to water, 1:1) per mouse.
[h] Administered as a solution of 2,4,5-T in 100% DMSO in a volume of 100 ul per mouse.
[i] 2,4,5-T was suspended in a honey solution (honey to water, 1:1) in a volume of 100 ul per mouse.
[j] $p = 0.01$.
[k] $p = 0.05$.

the same dose caused increased incidence of cleft palate and fetal mortality in both strains and cystic kidneys in C57BL/6 mice. Courtney et al. also reported increases in liver-to-body weight ratios in fetal mice.

These investigators also found that 4.6, 10, or 46.4 mg/kg 2,4,5-T given orally to Sprague-Dawley rats produced kidney anomalies and other embryotoxic effects at all levels (Table 17). The occurrence of hemorragic gastrointestinal tracts in rat fetuses was also reported.

Roll (125) found embryotoxic and teratogenic effects in NMRI mice after prenatal exposure to 2,4,5-T containing 0.05 ± 0.02 ppm dioxin (Table 18). 2,4,5-T at 20 to 130 mg/kg body weight was administered orally to the dams on each of days 6 to 15 of gestation. At 90 or 130 mg/kg, the percentage of resorptions and/or dead fetuses was markedly increased relative to the controls; however, maternal toxic effects were also observed at these dose levels.[12/] Statistically significant, dose-related reductions in fetal weight were observed at 20 mg/kg and above.

12/ Although the LD-50 for female NMRI mice had been previously determined to be 778 mg/kg, an increased maternal mortality rate was seen at 130 mg/kg and weight gain was depressed at doses above 60 mg/kg (125).

Table 17. Teratogenic Evaluation of 2,4,5-T[a] in Rats[b]

Test Animal	Route of Administration	Dose (mg/kg)	# Litters	Per Litter: Avg. # Live Fetuses	Per Litter: Abnormal Fetuses %	Per Litter: % Fetuses with: Enlarged Renal Pelvis	Per Litter: % Fetuses with: Cystic Kidney	Fetal Mortality %	Abnormal Litters %
Rats[c]									
Nontreated	----	---	7	9.9	9	9	0	11	43
Control	Stomach tube	[d]	14	8.7	12	12	<1	1	57
Treated	Stomach tube	4.6[e]	8	8.2	36[g]	18	21	12	88
Treated	Stomach tube	10.0[e]	7	7.1	46[g]	17	30[g]	28[f]	86
Treated	Stomach tube	46.4[e]	6	2.7	60[h]	27	33[h]	59[g]	67

[a] Contained approximately 30 ppm TCDD.
[b] Data from Courtney et al. (123).
[c] Treated from day 10 through 15 of pregnancy. Killed on day 20 of gestation.
[d] Dose, 200 ul honey solution (honey to water, 1:1) per rat.
[e] 2,4,5-T was suspended in a honey solution (honey to water, 1:1) in a volume of 200 ul per rat.
[f] p = 0.01.
[g] p = 0.05.
[h] The sample size was possibly too small to show a significant difference.

Table 18. Embryotoxic Effects of 2,4,5-T in NMRI Mice[a]

Dose mg/kg	Implantations per Pregnancy	Resorptions and/or Dead Fetuses No./Total No.	Resorptions and/or Dead Fetuses %	Fetal weight (grams)	Cleft Palate No./Viable No	Cleft Palate %
0	10.1	19/332	5.7	1.23	6/313	1.9
20	9.8	30/344	8.7	1.09	6/314	1.9
35	9.5	22/248	8.9	1.06	14/226	6.2
60	9.9	15/208	7.2	1.05	19/193	9.8
90	9.8	35/293	11.9	0.86	39/258	15.1
130	9.6	191/316	60.4	0.73	61/125	48.8

[a] Data from Roll (125).

Cleft palate increased among fetuses exposed to 35 mg/kg or more and was significant when compared with control values. Skeletal retardation effects, manifested as insufficient ossification, were also observed. The teratogenic no-effect level in mice for this 2,4,5-T was considered to be 20 mg/kg. Later studies with a specially prepared sample of 2,4,5-T with no detectable amounts of dioxin (detection limit: <0.02 ppm) confirmed these results in mice (125, 126). By contrast, daily oral administration of 25 to 150 mg/kg of either the dioxin-free or commercial grade 2,4,5-T (<0.1ppm dioxin) did not produce teratogenic effects in FW 49 rats (126).

Neubert and Dillman (127) also studied the effects of 2,4,5-T in NMRI mice, using three samples containing either (A) less than 0.02 ppm dioxin, (B) 0.05 ± 0.02 ppm dioxin (provided by Dr. Roll), or (C) an unknown amount of dioxin (Table 19). Their results confirmed those obtained by Roll (125). 2,4,5-T was administered to the dams orally in rape-seed oil on each of days 6 through 15 of gestation at 8 to 120 mg/kg body weight.

The average number of resorptions was significantly higher than the oil control at 60, 90, and 120 mg/kg of sample (A), and 90 mg/kg of samples (B) and (C). Total resorption of one litter was observed in four of the groups (30, 45, 60, and 90 mg/kg) treated with sample (A) and in

three of the litters treated with 90 mg/kg of sample (B); none was seen in the controls. Fetal weight was significantly depressed in all treated groups compared with the oil control.

The percentage of fetuses with cleft palate was significantly higher than the control group in all 2,4,5-T groups treated with 45 mg/kg or more. In the group treated with 120 mg/kg 2,4,5-T containing <0.02 ppm dioxin, 54% (7/13) of the litters and 11% (16/145) of the fetuses exhibited cleft palate compared with oil control values of 6% (4/65) and 0.7% (5/669), respectively.

These investigators also tested the butyl ester of 2,4,5-T and found similar effects. In experiments combining 2,4,5-T and TCDD, potentiation of teratogenic effects was observed. Sixty mg/kg of 2,4,5-T (sample A) combined with 0.3 ug/kg TCDD increased cleft palate frequency among fetuses from 5 to 14%. In this study no cleft palates were observed among fetuses treated only with 0.3 ug/kg TCDD.

Table 19. Embryotoxic Effects of 2,4,5-T[a/]

Treatment	Dioxin Content (ppm)	Dose (mg/kg)	Resorption (RES) % Litters with RES	Resorption (RES) % RES/Implantation Sites	Resorption (RES) RES/Single Litter w/RES (M)	Fetal Weight (grams)	Cleft Palate (CP) % Litters with CP	Cleft Palate (CP) % Fetuses with CP
None	---	---	24	4	0.6	1.26[b/]	6	0.6
Oil control	---	0.4 ml	32	4	0.5	1.30	6	0.7
2,4,5-T (A)	<0.02	8.0	35	3	0.4	1.27[b/]	<7	<1
		15.0	38	5	0.5	1.15[b/]	8	1
		30.0	56	7	0.8	1.09[b/]	11	1
		45.0	55	6	0.6	0.98[b/]	16	3[b/]
		60.0	63	11	1.2[b/]	1.01[b/]	20	5[b/]
		90.0	53	8	1.1[b/]	1.02[b/]	35	8[b/]
		120.0	54	10	1.3[b/]	0.95[b/]	54	11[b/]
2,4,5-T (B)	0.05	30.0	44	6	0.6	1.11[b/]	22	2
		60.0	57	7	0.4	1.11[b/]	71	9[b/]
		90.0	71	8	1.0[b/]	0.99[b/]	86	23[b/]
2,4,5-T (C)	unknown	90.0	71	13	1.4[b/]	1.00[b/]	72	26[b/]

a/ Data from Neubert and Dillman (127); 2,4,5-T sample (b) received from Roll (125).
b/ $p \leq 0.01$.

Sage et al. (132) injected NMRI mice subcutaneously with 50 and 110 mg/kg 2,4,5-T (<1.0 ppm dioxin) on each of days 6 through 14 of gestation. At 110 mg/kg, 2,4,5-T was teratogenic, causing fetal death, cleft palate, and other anomalies.

Courtney and Moore (128) studied the effects of 2,4,5-T in CD-1 random-bred mice, two strains of inbred mice, DBA/2J and C57BL/6J, and CD rats (Table 20).

Table 20. Embryotoxic Effects of Analytical and Technical 2,4,5-T[a/]

Species	Compound	Dose (mg/kg)	% Fetal Mortality per Litter	Fetal Weight (grams)	Cleft Palate (CP): % Litters Affected	Cleft Palate (CP): # CP per Affected Litter	Kidney Anomalies: % Litters Affected	Kidney Anomalies: # Affected Fetuses per Affected Litter
CD-1 Mouse Expt. 1	DMSO	---	6.6	1.35	0	0	0	0
	2,4,5-T (Tech.)	50	6.6	1.26	0	0	0	0
	2,4,5-T (Tech.)	100	7.5	1.00	33	3.0	0	0
	2,4,5-T (Tech.)	150[b/]	51.7	0.91	100	5.3	0	0
Expt. 2	DMSO	---	8.8	1.02	0	0	33	1.0
	2,4,5-T (Analy.)	100	9.6	0.73[d/]	89	4.4	78	1.7
Expt. 3	DMSO	---	8.4	1.09	0	0	63	2.0
	2,4,5-T (Tech.)	100	10.7	0.85[d/]	40	2.0	80	2.4
	2,4,5-T (Analy.)	100	11.6	0.86[d/]	40	2.0	100	4.2
	2,4,5-T (Analy.)	125	12.9	0.71[d/]	78	5.4	67	4.3
DBA/2 Mouse	DMSO	---	26.1	0.85	0	0	13	1.0
	2,4,5-T (Tech.)	100	27.0	0.67[d/]	27	1.0	9	1.0
C57B1/6 Mouse	DMSO	---	10.8	0.99	0	0	9	1.0
	2,4,5-T (Tech.)	100	15.9	0.75[d/]	40	1.2	0	0
CD Rat	Sucrose	---	3.4	2.48	0	0	0	0
	2,4,5-T (Tech.)	10	1.8	2.40	0	0	20	1.0
	2,4,5-T (Tech.)	21.5	1.4	2.54	0	0	38	1.3
	2,4,5-T (Tech.)	46.4	3.8	2.20	0	0	14	2.0
	2,4,5-T (Tech.)	80.0[c/]	52.1	2.30	0	0	50	4.0

a/ Data from Courtney and Moore (128).
b/ Investigators thought this data to be close to a maternal toxic dose.
c/ Maternal LD-40.
d/ $p < 0.05$.

2,4,5-T containing 0.5 ppm (technical) or 0.05 ppm (analytical) TCDD was administered subcutaneously to mice at 50 to 150 mg/kg in DMSO and orally to rats at 10 to 80 mg/kg in sucrose on each of days 6 to 15 of gestation. At 100 mg/kg or more, both 2,4,5-T samples produced significant reductions, which appeared to be dose related, in fetal weight in all strains of mice; rats were not affected. 2,4,5-T was fetocidal at two doses, but the investigators considered this effect to be due to maternal toxicity.

Both 2,4,5-T samples produced cleft palate in mice. For CD-1 dams treated with 100 mg/kg of either 2,4,5-T sample, 40% of the litters and two fetuses per affected litter evidenced cleft palate compared with 0% in the control (Expt. 3). No cleft palates were observed among the rat fetuses. To verify this observation, a second group of rats was given two 150 mg/kg doses of technical 2,4,5-T subcutaneously at the time of palate closure (days 13 to 14). Again, no cleft palates were observed; however, there was a significant increase in fetal mortality among treated animals (14%) when compared with the controls (0%).

Fetuses of CD-1 mice treated with analytical 2,4,5-T also showed increased incidences of kidney anomalies; the response to technical 2,4,5-T was not as great. At 100 mg/kg, 100% of the litters and 4.2 fetuses per affected litter of dams treated with analytical 2,4,5-T displayed

kidney anomalies, compared with 80% and 2.4 for technical 2,4,5-T and 63% and 2.0 for controls (Expt. 3). The effect in inbred strains of mice was comparable with control values. In rats, technical 2,4,5-T at all dose levels produced higher incidences of litters affected and numbers of fetuses per litter affected than seen in the control animals. The maximum effects on kidney anomalies in rats were 50% of the litters and 4.0 fetuses per litter at 80 mg/kg, compared with 0% in the control litters.

In another study using CD-1 mice, Courtney (134) administered 0.45 to 1.0 mM/kg body weight per day of 2,4,5-T (0.05 ppm dioxin) either orally or subcutaneously during various segments of the gestation period (Table 21).[13/] Cleft palate was seen in all groups treated with 2,4,5-T; there were no instances of this anomaly within the control groups. At 0.8 mM/kg, 48% of total fetuses and 37% of the litters evidenced this malformation. Statistically significant ($p \leq 0.05$) increases in the percentage of fetuses dead and/or resorbed were observed at the highest doses. All dose levels had adverse effects on fetal weight. The author noted that by slightly altering experimental conditions, the cleft palate effect and the effects on fetal mortality and fetal weight could be produced independently.

13/ Maternal toxicity was also observed, evidenced by reductions in maternal weight gain and increased liver-to-body weight ratios (134).

Table 21. Embryotoxic Effects of 2,4,5-T in CD-1 Mice[a/]

Vehicle	Dose (mM/kg)	Days Dosed	Viable Normal Fetuses #/total #	Viable Normal Fetuses %	% Fetal Mortality	Fetal Weight (grams)	Cleft Palate (avg. %) Fetuses	Cleft Palate (avg. %) Litters
oil:Ac[b/]	---	10-15	75/80	94	6	0.95	---	---
	---	11-13	99/112	88	11	0.94	---	---
	---	12-15	108/126	86	13	1.01	---	---
	0.45	10-15	86/107	80	17	0.89	7	6
	0.80	11-13	88/122	72	14	0.87	16	14
	0.80	11-14	21/59	36	29[e/]	0.87	48	37
	1.00[d/]	12-15	75/82	91	8	0.96	1	1
DMSO[c/]	---	12-15	152/171	89	12	1.03	---	---
	1.00	12-15	11/68	16	72[f/]	0.70	48	67

a/ Data from Courtney (134).
b/ Corn oil:Acetone (9:1)--oral.
c/ Dimethylsulfoxide -- subcutaneous.
d/ This concentration exceeded the solubility characteristics of the vehicle. Doubling the volume of vehicle resulted in effects more consistent with those found at lower doses.
e/ $p \leq 0.05$.
f/ $p \leq 0.001$.

Khera and McKinley (130) studied the prenatal and postnatal effects of 2,4,5-T in Wistar rats, using four samples containing no TCDD (detection limit: 0.5 mg/kg) [Table 22]. Twenty-five to one hundred fifty mg/kg body weight per day were administered to the dams, orally in gelatin or corn oil, on days 6 to 15 of gestation. At 25 and 50 mg/kg, the differences between experimental and control values were minimal. However, at 100 and 150 mg/kg, there were significant ($p < 0.05$) effects on fetal weight, number of dead fetuses, and percentage of malformed fetuses

per litter.[14/] The larger proportion of malformed fetuses in the treated groups resulted from either an increased incidence of skeletal anomalies also seen in the controls or a low incidence of abnormalities not observed in the controls. The former category included wavy ribs, retarded ossification, extra ribs, and a variety of sternal defects; the latter included fused ribs, small-sized distorted scapula, malformed humerus shaft, and bent radius or ulna. Abnormal kidneys were observed in 7 to 45% of the examined fetuses treated with sample T-1, compared with a control value of 20 to 35%.

Table 22. Effects of 2,4,5-T on Wistar Rat Fetuses[a/]

Compund	Dose (mg/kg)	# of Litters	Avg. # per Litter: Viable Fetuses	Dead Fetuses	Fetal Weight (grams)	Avg. % Malformed Fetuses per Litter[b/]
T-1	Treated Control	14	11.1	0.6	4.65	15
	50	7	12.9	1.3	4.84	24
	100	9	11.3	1.9	4.60	29
T-2	Treated Control	10	9.2	0.6	5.34	10
	25	13	10.5	0.8	5.06	15
	50	12	11.7	0.5	5.15	9
	100	9	8.6	2.4	4.57	32
	Treated Control	10	12.6	0.7	4.67	26
	25	11	12.7	0.5	5.15	10
	50	14	11.5	1.4	4.91	28
	100	10	11.0	0.6	4.35	36
	150	5	11.6	2.2	3.98	56
T-3	Treated Control	10	11.8	0.7	5.31	17
	25	14	12.2	0.9	5.00	11
	50	2	11.0	0.5	4.75	56
	100	12	12.6	0.9	5.00	37
	150	1	11.0	1.0	3.00	91
T-4[c/]	50	8	11.3	1.1	4.94	14

a/ Data from Khera and McKinley (130).
b/ One or more skeletal malformation (viable fetuses).
c/ No treated control given.

14/ Statistical significance was determined using the average value per dose level. Data from T-4 were not used in this analysis.

In the postnatal portion of the study, after normal delivery, survival rate, sex ratio, and pup weight on days 1 and 21 were compared. Although treated pups surviving from day 2 to 21 were slightly smaller at some dose levels, there were no significant differences from controls for any variable. In some experiments, litters were standardized at 8 pups on day 2, and the remaining littermates examined for defects. The increased incidences of malformations among treated groups were comparable to those found in the prenatal study. Assuming the same incidence for pups not examined, the investigators concluded that there were no real differences in survival rates among control and treated groups. The butyl ester of 2,4,5-T produced similar toxic effects.

Sokolik (131) orally administered 100 and 400 mg/kg and 50 and 200 mg/kg of 2,4,5-T and its butyl ester to rats of the Rappolovo line on each of days 1 to 14 or 1 to 16 of pregnancy. At 100 mg/kg, 2,4,5-T produced embryos with a combination of deformities including absence of lower jaw, abnormal hind limbs, and exophthalmos. At 400 mg/kg, the embryos of treated rats evidenced cleft palate, hydrocephalus, hydronephrosis, and abnormalities of the upper limbs which included tridactyly, webbed toes, and abnormal shortness.

The butyl ester of 2,4,5-T was more toxic than the parent compound, causing more than 30% embryonic mortality at 200 mg/kg. The lower dose, 50 mg/kg, also caused high mortality among the embryos. Cleft palate, hydronephrosis, hydrocephalus, and extensive gastrointestinal hemorrhages were also observed within the treated groups. From these results, the author concluded that 2,4,5-T and its derivatives have a high potential for teratogenic activity.

Collins and Williams (124) tested seven samples of 2,4,5-T from different sources for embryotoxic effects in golden Syrian hamsters (Mesocricetus auratus) [Table 23]. The dioxin contents ranged from not detectable (detection limit < 0.1 ppm) to 45 ppm. Daily oral doses of 20 to 100 mg/kg body weight were administered in acetone:corn oil:carboxymethyl cellulose (1:5.8:10) on days 6 to 10 of gestation. 2,4,5-T with no detectable dioxin significantly ($p < 0.05$) reduced fetal weight and fetal viability per litter at all levels tested.

Total fetal mortality was greatly increased at all levels when compared with controls and was dose-dependent, as was the effect on fetal viability. The increased incidence of gastrointestinal hemorrhage also appeared to be dose related. At 100 mg/kg, "pure" 2,4,5-T caused increased incidences of malformations and reductions in the number of live fetuses per litter. One "pure" sample, F, at 100 mg/kg significantly reduced fetal weight from 1.8

Table 23. Embryotoxic Effects of 2,4,5-T in Hamsters[a]

Compound	Dioxin Content (ppm)	Dose (mg/kg)	Fetuses: % Total Mortality	Fetuses: Avg # Live per Litter	Fetuses: Avg Weight[b] (grams)	% Fetal Viability per Litter	% Abnormalities per Live Litter	% Hemorrhages per Total Live Fetuses
Control	---	---	3.4	11.0	1.8	96.7	3.5	0.32
A	45	20	32.3	7.3	1.7	68.1[d]	25.0[d]	28.4
		40	74.3	3.7	1.7	25.8[d]	33.3[d]	75.7
		80	94.4	0.8	1.6	5.3[d]	100.0[d]	42.9
		100	100.0	0	---	0[d]	---	---
B	2.9	40	7.2	9.1	1.7	93.1	0	0
		80	9.8	10.4	1.7	90.1[d]	12.5	2.4
		100	11.4	12.8	1.7	88.5[d]	50.0[d]	13.0
C	0.5	20	8.5	12.6	1.7[d]	90.8[d]	0	8.6
		40	4.0	13.4	1.6[d]	95.9	11.1	2.5
		80	43.6	6.6	1.6[d]	58.3[d]	40.0[d]	12.5
		100	57.2	5.1	1.5[d]	40.2[d]	40.0[d]	7.6
D	0.1	40	2.4	11.4	1.8	97.8	0	0
		80	33.3	7.8	1.7	68.3[d]	0	2.1
		100	47.1	6.0	1.6[d]	57.2[d]	0	5.6
E	ND[c]	40	10.7	11.2	1.5[d]	88.2[d]	0	1.5
		80	29.9	8.7	1.5[d]	69.1[d]	0	4.2
		100	56.3	6.3	1.5[d]	53.1[d]	36.4[d]	0
F	ND	100	31.3	7.3	1.6[d]	71.4[d]	40.0[d]	6.8
G	ND	100	30.0	8.4	1.6[d]	68.7[d]	0	16.7

[a] Data from Collins and Williams (124).
[b] Apparently normal weights for samples A and B attributed to edema.
[c] Not detected.
[d] $p < 0.05$.

to 1.6 grams, reduced fetal viability from 96.7 to 71.4%, and increased abnormalities from 3.5 to 40%. The anomalies associated with 2,4,5-T containing no dioxin were exencephaly, eye abnormalities, delayed head ossification, and hind limb deformities.

Increasing the level of dioxin contamination increased fetal mortality and the incidence of abnormalities per litter; fetal viability was reduced. A clear correlation was found between the level of dioxin and abnormalities per litter. Although the incidence of hemorrhages also increased, no relationship between it and dioxin level could be found. Bulging eyes (absence of eyelid) and delayed ossification were the most common anomalies seen among fetuses exposed to dioxin-contaminated 2,4,5-T; exencephaly, edema, cleft palate, ectopic heart, and fused ribs were also observed.

Emerson et al. (141) found no adverse effects of commercial 2,4,5-T, containing 0.5 ppm TCDD, on fetal development in Sprague-Dawley derived rats and New Zealand white rabbits. Daily oral doses of 2,4,5-T in gelatin were administered to the rats at 1 to 24 mg/kg on days 6 to 15 of gestation; to the rabbits at 10 to 40 mg/kg on days 6 to 18 of gestation. The investigators found no maternal or embryonic toxic effects in either species, nor was 2,4,5-T

considered teratogenic under the conditions of these experiments. The most frequently observed abnormalities were accessory ribs, hydronephrosis, and retardation in the development of the sternebrae. With the exception of partially ossified sternebrae in both species and bilateral accessory ribs in the rabbit, the incidence of these anomalies was greater in the control animals than in the examined treated groups.

Sparschu et al. (140) orally administered 2,4,5-T, containing 0.5 ppm TCDD, to rats in daily doses of 50 and 100 mg/kg on days 6 to 15 and 6 to 10 of gestation, respectively. Results are given in Table 24. At 50 mg/kg, there were no significant maternal or embryonic toxic effects attributable to 2,4,5-T except for an increased incidence of delayed skull ossification, and a single fetus with intestinal hemorrhage. At 100 mg/kg, 2,4,5-T was toxic to both dams and fetuses.[15/]

15/ The high rate of maternal mortality caused dosing to be stopped on day 10, instead of day 15. Significant reductions in weight gain were also observed.

Table 24. Effects of 2,4,5-T on Fetal Development of Rats[a]

Parameter	Dose (mg/kg per day) 0	50	100
# Viable fetuses			
Total	252	203	13[b]
Mean per litter	11	11	---
% Resorptions			
Litters	68	61	100
Total fetuses	6.7	12.1	75[c]
Fetal weight (grams)			
Male	4.41	4.38	3.57[c]
Female	4.17	4.15	3.52[c]
Sex Ratio (M:F)	53:47	44:56	23:77
Abnormalities (% fetuses examined)			
Poorly ossified sternebrae			
Fifth	15.2	22.1	57.1[c]
Second and fifth	3.0	4.2	14.3
Multiple	8.3	12.6	14.3
Malaligned sternebrae	0.8	2.1	28.6[c]
Delayed ossification			
Interparietal	3.8	16.8[c]	28.6[c]
Parietals	3.0	16.8[c]	57.1[c]
Frontals	0.8	7.4[c]	14.3

a/ Data from Sparschu et al. (140).
b/ All viable fetuses from one litter.
c/ $p < 0.05$

Resorptions were observed in all litters; 75% were totally resorbed. Fetal weight was significantly ($p < 0.001$) reduced in both sexes and the sex ratio was shifted in favor of females. Abnormalities observed which had significantly ($p < 0.05$) higher incidences than in the

controls were poorly ossified and malaligned sternebrae and delayed skull ossification. The investigators concluded that the delayed ossification observed in this study was a reversible manifestation, rather than a true teratogenic effect.

(b) Adverse Reproductive Effects in Other Mammalian Test Systems

Adverse reproductive effects of 2,4,5-T exposure have been observed in other mammalian test systems. Lloyd et al. (173) reported on *in vivo* enzymatic studies showing reduced uptake and metabolism of testosterone by the prostate gland in male mice treated orally with doses of 2,4,5-T (6.25, 12.5, or 25 mg/kg, ten times daily).

Yefimenko (151) reported on the effects of acute and chronic exposure to the butyl ester of 2,4,5-T on gonadal and somatic tissue in an *in vivo* cytogenetic study in male albino rats. Chronic effects on the gonads were observed after exposure to 0.1 ug/kg for two and one half months. Adverse effects (seen at seven months, when the experiment was terminated), which were considered persistent effects, included testicular atrophy, decreased sperm count, desquamated tubules, and aberrant cells in the germinal epithelium. Chromosomal aberrations were also observed during the chronic phase of the experiment. EPA evaluation of this study found inadequacies in the methodology which would prevent the drawing of firm conclusions from this data (106).

Recent studies in rats by Sjoden and Soderberg [cited in (25)] appear to show that prenatal exposure to 2,4,5-T leads to behavioral abnormalities and changes in thyroid activity and brain seritonin levels in the progeny. Single oral doses of 100 mg/kg were administered to the dams on days 7, 8, or 9 of pregnancy.

(c) Adverse Effects in Avian Species

Embryotoxic effects in avian species due to 2,4,5-T exposure have been reported. Verrett (136) studied the effects of 2,4,5-T, containing either 27 or 0.5 ppm TCDD, on chicken eggs. The 2,4,5-T was injected through the air cell of the eggs, either preincubation or on the fourth day of incubation. The sample containing 27 ppm TCDD was found to be more lethal (LD-50 = 25 ug/egg) than the less contaminated sample (LD-50 = 100 ug/egg). Both samples produced teratogenic effects, including chick edema, eye defects, beak defects (primarily cleft palate), and short, twisted feet resulting from tendon slippage. Teratogenic effects were observed at doses as low as 1 ppm (50 ug/egg) with the sample containing 0.5 ppm TCDD and as low as 0.125 ppm (6.25 ug/egg) with the sample containing 27 ppm TCDD.

Lutz and Lutz-Ostertag (138) studied the action of 2,4,5-T, in aqueous solution at a concentration of 2 to 10 g/liter, on the embryonic development of quail (Coturnix coturnix japonica), chicken (Gallus gallus),

pheasant (*Phasianus colchicus*), and two partridge species (*Alectoris rufa* and *Perdrix perdrix*). The 2,4,5-T was administered by dipping, spraying, and organo-typic cultures. Abnormal genital tracts were observed in all species, indicating abnormal sexual differentiation. Further, morphological changes in the testes often gave the appearance of true testicular atrophy. In another study, 2,4,5-T affected fertility in birds of both sexes (139).

(d) Studies in Avian Species in Which Adverse Effects Were Not Observed

Using 2,4,5-T contaminated with less than 0.1 ppm dioxin, Strange and Kerr (142) found no abnormal development in chicken embryos. Doses of 12.5, 25, 50, 75, 100, and 125 mg/kg were injected into eggs on days 0 and 5 of incubation; observations were made 48 hours later. At this developmental stage, kidneys were not sufficiently developed to detect the tubule lesions reported by Bjorklund and Erne (143).

(e) Summary

Studies have established that 2,4,5-T is fetotoxic and teratogenic at doses as low as 35 mg/kg (0.05 ± 0.02 ppm TCDD) in mice (125); 4.6 mg/kg (approximately 30 ppm TCDD) in rats (123); and 20 mg/kg (0.5 ppm TCDD) in hamsters (124). Cleft palate and kidney anomalies have been observed in mice, rats, and hamsters. No fetotoxic or teratogenic effects (no-effect levels) have been observed at doses of 20 mg/kg (0.05 ± 0.02 ppm TCDD) in mice (125) and 25 to 150 mg/kg (0.05 ± 0.02 ppm TCDD) in rats (125).

(3) Exposure Analysis

In order to determine whether a rebuttable presumption should be issued based on reproductive and fetotoxic effects, pursuant to Section 162.11(a)(3)(ii)(B), the Working Group must determine whether or not an ample margin of safety

exists between the levels of 2,4,5-T and/or TCDD which produce reproductive and fetotoxic effects, and the level(s) to which humans can reasonably be anticipated to be exposed.

The cancellation of uses of 2,4,5-T on food crops intended for human consumption and for use around the home, recreation sites, aquatic areas, and ditch banks in 1970 was thought to have eliminated the potential exposure to that portion of the population at risk (women of child-bearing age).

Social changes over the last few years, however, have given women the opportunity for employment in areas that once were considered open only to men. Since women of child-bearing age are now employed in occupations such as pesticide applicators, operators of highway construction and maintenance equipment, foresters, and chemical formulators, they have become part of the population at risk with potential exposure to 2,4,5-T and/or TCDD.

In order to determine whether an ample margin of safety exists, the Working Group must first determine how much 2,4,5-T a woman could be exposed to through oral, dermal, or inhalation exposure. For each of these analyses, the Working Group assumes a woman to weigh 60 kg. The following calculations are based on an exposure analysis for 2,4,5-T and TCDD performed by EPA's Criteria and Evaluation Division [CED] (164).

(a) Oral Exposure

For purposes of this analysis, the Working Group considered currently registered uses where the possibility of oral exposure to 2,4,5-T and/or TCDD existed. Treatment of range and pasture land could result in oral exposure through ingestion of meat and milk from animals grazing on the treated area. Since actual data on residues of 2,4,5-T in animals grazing on treated rangeland is

unavailable, for purposes of the 2,4,5-T oral exposure analysis, the Working Group used residue information obtained in a feeding study (37) in which cattle were fed considerably higher amounts of 2,4,5-T than they would normally be exposed to in grazing on treated land. The following calculations are based on the average quantities of food eaten per day (1.5 kg), as reported by Lehman (144, 165).

Table 25. 2,4,5-T Oral Exposure Analysis

	Whole Milk	Meat (Beef)
No-adverse-effect level for teratogenicity in mice	20 mg/kg	20 mg/kg
Average level of 2,4,5-T identified	0.103 ppm[a/]	0.2 ppm[a/]
% of food item in total human diet	19.6%	4.6%
Average amount of food eaten per day	1.5 kg	1.5 kg
Exposure to 2,4,5-T per day	0.0005 mg/kg	0.0002 mg/kg

a/ Animals were fed at 300 ppm 2,4,5-T in the diet for 2 to 3 weeks. This is a worst case assumption for cows grazing on freshly-treated pasture without a withdrawal period; all milk and meat was obtained from such cows. Meat (beef) includes muscle, fat, and liver tissues which constitute the major portion of edible meat.

To find the average daily intake of a single food item, multiply the average daily food intake by the percent of that item in the total diet: for milk, 1.5 kg X 19.6% = 0.294 kg; and for meat (beef), 1.5 kg X 4.6% = 0.069 kg.

The quantity of 2,4,5-T in the average daily diet equals the average daily intake of each food item multiplied by the level of 2,4,5-T in the food item: for milk, 0.294 kg X 0.103 ppm = 0.03 mg; and for meat (beef), 0.069 kg X 0.2 ppm = 0.014 mg.

The theoretical exposure of an average woman equals the amount of 2,4,5-T in the daily diet divided by the weight of the average woman: for milk, 0.03 mg / 60 kg = 0.0005 mg/kg; and for meat (beef), 0.014 mg / 60 kg = 0.0002 mg/kg; total exposure from milk and beef products could be 0.0007 mg/kg per day.

Existing data on TCDD residues in animals grazing on treated rangeland are too meager to use for an analysis of TCDD exposure to humans through ingestion of meat or milk from animals so exposed.

The Working Group considers that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and the calculated oral exposure level for 2,4,5-T (0.0007 mg/kg per day) does constitute an ample margin of safety. Since this risk criterion for other chronic adverse effects has not been met or exceeded, a rebuttable presumption does not arise.

(b) Dermal Exposure

In order to conduct these analyses, the Working Group must determine the amount of 2,4,5-T and/or TCDD which would come in contact with the skin and the amount that would be absorbed.

(i) Spray Applicator: Back-pack Sprayer

For purposes of this analysis, the Working Group assumes the applicator to be a 60-kg woman of child-bearing age, and the site of application either a right-of-way or spot treatment of pasture or rangeland. The equipment is a back-pack sprayer (166). The following calculations of exposure are based on dilution for spraying of three pints of formulated product per 32 pints of water. Typical 2,4,5-T formulations, based on inspection of a large number of registered labels (164), range from 4 to 6 pounds active ingredient (acid equivalent) per gallon. The product used in this exposure analysis has an assumed concentration of 4 pounds 2,4,5-T per gallon. Label recommendations vary from a recommended dilution of 0.094 to 4 pounds acid equivalent per 32 pints of water. A dilution rate of 1.6 pounds per 32 pints has been selected as representative of a typically-used spray mixture.

Wolfe et al. (166) studied dermal exposure to fenthion during hand back-pack spraying for mosquitoes

for ten situations. Exposure ranged from 0.1 to 6.3 mg/hr, with a mean value of 3.6 mg/hr (6 ml/hr). Method of application was a hand pressure sprayer, using a 0.06% spray. Workers wore short-sleeved, open-necked shirts with no gloves or hat. Based on Wolfe's data, CED (164) calculated a dermal exposure of approximately 0.177 pints per day. CED (164) also determined that approximately 10% of the 2,4,5-T and TCDD coming in contact with the skin of the applicators would be absorbed even after washing, based on absorption studies with other pesticides (145, 146, 163).

Table 26. Back-pack Sprayer Dermal Exposure Data

	2,4,5-T	TCDD
Use Dilution rate —	3 pints (1.6 pounds 2,4,5-T) per 32 pints water	3 pints (0.00000016 pounds TCDD) per 32 pints water
Amount of diluted material gotten on skin daily	0.18 pint	0.18 pint
% Diluted material absorbed	10%	10%
Exposure level	409 mg	0.0409 ug
Dose level	6.8 mg/kg	0.0007 ug/kg
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

The following calculations (see Table 27 for mathematics) will give the daily dermal exposure for both 2,4,5-T

and TCDD: 1) convert the dilution rate to grams; 2) multiply this figure by 1,000 (for 2,4,5-T) to convert to milligrams and by 1,000,000 (for TCDD) to convert to micrograms; 3) multiply this figure by the daily dermal dose of diluted material; 4) multiply this figure by the percent absorbed; and 5) divide this figure by the weight of the applicator for the daily exposure to 2,4,5-T or TCDD per 8-hour working day.

Table 27

2,4,5-T	TCDD
1) 1.6 pounds/32 pt X 454 g/pound = 22.70 g/pt;	1) 0.00000016 pounds/32 pt X 454 g/pound = 0.00000227 g/pt;
2) 22.70 g/pt X 1,000 mg/g = 22,700 mg/pt;	2) 0.00000227 g/pt X 1,000,000 ug/g = 2.27 ug/pt;
3) 22,700 mg/pt X 0.18 pt = 4,086 mg;	3) 2.27 ug/pt X 0.18 pt = 0.41 ug;
4) 4,086 mg X 10% = 408.6 mg	4) 0.41 ug X 10% = 0.041 ug;
5) 408.6 mg / 60 kg = 6.8 mg/kg per day	5) 0.041 ug / 60 kg = 0.0007 ug/kg per day

The Working Group considers that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (6.8 mg/kg), as well as the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 ug/kg) and this calculated exposure level for TCDD (0.0007 ug/kg), do not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide

products containing 2,4,5-T and/or TCDD pursuant to 40 CFR Section 162.11(a)(3)(ii)(B).

(ii) Spray Applicator: Tractor-mounted, Low-boom Spray Equipment

For the purpose of this analysis, the Working Group assumes the applicator to be a 60-kg female of child-bearing age clearing brush on either rangeland or rights-of-way. The same product cited above (2,4,5-T at 4 pounds/gal) is being used, and the dilution rate is 1.6 pounds of formulation to 32 pints of water (equal to 4 pounds of 2,4,5-T per 10 gallons of water). Based on exposure studies using similar equipment but a different herbicide (147), the Working Group determined that, during an eight-hour working day, the applicator would get 0.048 pints of diluted material on her skin. The Working Group determined that 10% of the pesticide on the skin would be absorbed (145, 146, 163).

Table 28. Dermal Exposure Data (Tractor Mounted Equipment)

	2,4,5-T	TCDD
Use Dilution rate	3 pints (1.6 pounds 2,4,5-T) per 32 pints water	3 pints (0.00000016 pounds TCDD) per 32 pints water
Amount of diluted material gotten on skin daily	0.048 pint	0.048 pint
% Diluted material absorbed	10%	10%
Exposure level	109 mg	0.0109 ug
Dose level	1.8 mg/kg	0.00018 ug/kg
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

The following calculations (see Table 29 for mathematics) will give the daily dermal exposure for both 2,4,5-T and TCDD: 1) convert the dilution rate to grams; 2) multiply this figure by 1,000 (for 2,4,5-T) to convert to milligrams and by 1,000,000 (for TCDD) to convert to micrograms; 3) multiply this figure by the daily dermal dose of diluted material; 4) multiply this figure by the percent absorbed; and 5) divide this figure by the weight of the applicator for the daily exposure to 2,4,5-T or TCDD per 8-hour working day.

Table 29

2,4,5-T	TCDD
1) 1.6 pounds/32 pt X 454 g/pound = 22.70 g/pt;	1) 0.00000016 pounds/32 pt X 454 g/pound = 0.00000227 g/pt;
2) 22.70 g/pt X 1,000 mg/g = 22,700 mg/pt;	2) 0.00000227 g/pt X 1,000,000 ug/g = 2.27 ug/pt;
3) 22,700 mg/pt X 0.048 pt = 1,089.6 mg;	3) 2.27 ug/pt X 0.048 pt = 0.109 ug;
4) 1,089.6 mg X 10% = 108.96 mg;	4) 0.109 ug X 10% = 0.011 ug;
5) 108.96 mg / 60 kg = 1.8 mg/kg per day	5) 0.011 ug / 60 kg = 0.00018 ug/kg per day

The Working Group considers that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (1.8 mg/kg), as well as the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 ug/kg) and this calculated exposure level for TCDD (0.00018 ug/kg), do not constitute an

ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T and/or TCDD pursuant to 40 CFR Section 162.11(a)(3)(ii)(B).

(iii) Aerial Application: Exposed Population Directly Beneath Spray Plane

Caplan et al. (167), working with aerially applied malathion in oil sprays applied at 0.46 pounds per 0.76 gallons water/acre, determined a dermal exposure to persons directly beneath the spray plane for bare skin (head, neck, shoulders, forearms, hands, and thighs) of 3.556 mg/day. With these data, an equivalent dermal exposure for 2,4,5-T and TCDD, aerially applied at 4 pounds acid equivalent 2,4,5-T per 10 gallons water/acre, can be determined.

Table 30. Dermal Exposure Data (Aerial Application)

Dermal exposure to aerially applied malathion	3.556 mg/0.46 pounds malathion per acre	
	2,4,5-T	TCDD
Use Dilution rate	4 pounds 2,4,5-T per 10 gallons of water/acre	0.0000004 pounds TCDD per 10 gallons of water per acre
% Diluted material absorbed	10%	10%
Exposure level	3.1 mg	0.0003 ug
Dose level	0.051 mg/kg	5×10^{-6} ug/kg
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

The following calculations (see Table 31 for mathematics) will give the daily dermal exposure for both 2,4,5-T and TCDD: 1) divide the dermal exposure to malathion by the malathion application rate and multiply by the application rate of 2,4,5-T and TCDD to obtain the dermal exposure; for TCDD, multiply this figure by 1,000 to convert to micrograms; 2) multiply this figure by the percent absorbed; and 3) divide this figure by the weight of the applicator for the daily exposure to 2,4,5-T or TCDD per 8-hour working day.

Table 31

2,4,5-T	TCDD
1) 3.556 mg/0.46 pounds X 4 pounds = 31 mg;	1) 3.556 mg/0.46 pounds X 0.0000004 pounds = 0.000003 mg X 1,000 = 0.003 ug;
2) 31 mg X 10% = 3.1 mg;	2) 0.003 ug X 10% = 0.0003 ug;
3) 3.1 mg/ 60 kg = 0.051 mg/kg per day	3) 0.0003 ug / 60 kg = 5×10^{-6} ug/kg per day

The Working Group considers that the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 ug/kg) and this calculated dermal exposure level for TCDD (5×10^{-6} ug/kg) does constitute an ample margin of safety. The Working Group also considers, however, that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (0.051 mg/kg)

does not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T pursuant to 40 CFR Section 162.11(a)(3)(ii)(B).

(c) Inhalation Exposure: Aerial Application

There are no studies available on inhalation exposure of 2,4,5-T. There are, however, several studies on inhalation exposure to malathion (167, 168) which CED used as a model for this 2,4,5-T exposure analysis (164). Caplan et al. (167) determined an air concentration, for unprotected persons directly beneath the spray plane during application and for two hours afterward, of 0.067 mg malathion/m^3 from aerial application of 0.46 pounds AI/gallon per acre. The collection period spanned the course of the actual application time plus two hours thereafter. The authors considered the sampling technique to be equivalent to average inspiration through the nostrils. This inhalation exposure (amount available for inhalation) was 12% of the applied malathion. Caplan et al. further reported that the average median diameter (= volume median diameter, or vmd[16/]) was 109 microns. Based on work by Akesson and Yates (168), CED (164) estimated that the size of the malathion droplets which could be inhaled was

16/ The vmd is that droplet size which divides the total volume of drops in half, i.e., 50% of the volume is in drops above the vmd size and 50% below it.

under 60 microns. Since 2,4,5-T is typically applied as a medium or coarse spray, while malathion is applied as a fine spray, the percent of 2,4,5-T droplets small enough to be inhaled (under 60 microns) would be less than the percent of malathion droplets small enough to be inhaled. According to Akesson and Yates (168), 2% of 2,4,5-T spray droplets would be available for inhalation (or 1/6 the amount of malathion droplets available for inhalation), on a "worst case" basis.

Table 32. Inhalation Exposure Data (Aerial Application)

Air concentration of aerially applied malathion	0.067 mg/m^3 with application rate of 0.46 pounds malathion per gallon per acre	
	2,4,5-T	TCDD
Use Dilution rate	4 pounds 2,4,5-T per 10 gallons of water/acre	0.0000004 pounds TCDD per 10 gallons of water per acre
Lung Absorption Rate	100%	100%
Breathing Rate	1.8 m^3/hr	1.8 m^3/hr
Exposure level	0.34 mg per 2 hr	0.000032 ug per 2 hr
Dose level	0.023 mg/kg per 8 hr	2 X 10^{-6} ug/kg per 8 hr
No-Adverse-Effect level for teratogenic effects	20 mg/kg	0.03 ug/kg

The following calculations (see Table 33 for mathematics) will give the daily inhalation exposure for both 2,4,5-T and TCDD: 1) multiply the air concentration of malathion by the amount of 2,4,5-T and TCDD applied, then multiply this figure by 1/6 for the inhalation exposure to 2,4,5-T and TCDD; for TCDD, multiply this figure by 1,000 to convert to micrograms; 2) multiply this figure by the breathing rate; 3) multiply this figure by eight [8] to get the 8-hour exposure total; and 4) divide this figure by the weight of the applicator for the inhalation exposure to 2,4,5-T or TCDD per 8-hours exposure.

Table 33.

2,4,5-T	TCDD
1) 0.067 mg/cu m per 0.46 pounds X 4 pounds = 0.58 mg/cu m X 1/6 = 0.097 mg/cu m;	1) 0.067 mg/cu m per 0.46 pounds X 0.0000004 pounds = 0.000000058 mg/cu m X 1/6 = 0.000000009 mg/cu m X 1,000 = 0.000009 ug/cu m;
2) 0.097 mg/cu m X 1.8 cu m/-hr = 0.17 mg/hr;	2) 0.000009 ug/cu m X 1.8 cu m/hr = 0.000016 ug/hr;
3) 0.17 mg/hr X 8 = 1.36 mg;	3) 0.000016 ug/hr X 8 = 0.000128 ug;
4) 1.36 mg / 60 kg = 0.026 mg/kg exposure per day	4) 0.000128 / 60 kg = 2×10^{-6} ug/kg per day

The Working Group considers that the difference between the no-adverse-effect level of TCDD for teratogenic effects (0.03 ug/kg) and this calculated dermal exposure level for TCDD (2×10^{-6} ug/kg) does constitute an ample margin of safety. The Working Group also considers,

however, that the difference between the no-adverse-effect level of 2,4,5-T for teratogenic effects (20 mg/kg) and this calculated dermal exposure level for 2,4,5-T (0.026 mg/kg[17]) does not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T pursuant to 40 CFR Section 162.11(a)(3)(ii)(B).

(d) Cumulative Exposure

The Working Group has also considered the possibility of a single individual being exposed through two or more of the above routes. The results (derived from Tables 27, 29, and 31) are shown in Table 34. The Working Group also notes that possible cumulative exposure to several dioxin-containing pesticides could increase the total body burden and increase total risk from dioxin exposure.

The Working Group considers that the differences between the no-adverse-effect level of TCDD for teratogenic effects (0.03 ug/kg) and the calculated cumulative exposure levels for TCDD in Situations 2 and 3 (see Table 34) do constitute an ample margin of safety. The Working

17/ Johnson (63) [see Section I.G.(3)], in a review article, calculated a daily inhalation exposure to phenoxy herbicides of 0.025 ug/kg for a 70-kg adult. The calculations were based on actual air monitoring data of air samples collected in two wheat-growing areas in the state of Washington during spring and summer and analyzed for phenoxy herbicides. The author did not specify how soon after application the samples were taken.

Table 34. Cumulative Exposure to 2,4,5-T and TCDD

Situation #1: 2,4,5-T	Situation #1: TCDD
Oral- 0.0007 mg/kg	Oral- -----
Dermal- 6.8 mg/kg	Dermal- 0.0007 ug/kg
Inhal.- 0.2 mg/kg[a/]	Inhal.- negligible[a/]
Cum. = 7.0 mg/kg	Cum. = 0.0007 ug/kg
Situation #2: 2,4,5-T	**Situation #2: TCDD**
Oral- 0.0007 mg/kg	Oral- -----
Dermal- 1.8 mg/kg	Dermal- 0.00018 ug/kg
Inhal.- 0.05[a/]	Inhal.- negligible[a/]
Cum. = 1.85 mg/kg	Cum. = 0.00018 ug/kg
Situation #3: 2,4,5-T	**Situation #3: TCDD**
Oral- 0.0007 mg/kg	Oral- -----
Dermal- 0.051 mg/kg	Dermal- 5×10^{-6} ug/kg
Inhal.- 0.026 mg/kg	Inhal.- 2×10^{-6} ug/kg
Cum. = 0.0777 mg/kg	Cum. = 7×10^{-6} ug/kg

a/ Calculations were made on a worst-case basis as 3% of dermal exposure based on Wolfe (179) who states, "over 97% of the pesticide to which the body is subjected during most exposure situations, and especially to applicators of liquid sprays, is deposited on the skin." TCDD inhalation exposure values were negligible: Situation #1, 21×10^{-6} ug/kg; Situation #2, 54×10^{-7} ug/kg.

Group also considers, however, that the differences between the no-adverse-effect levels of 2,4,5-T and TCDD for teratogenic effects (20 mg/kg and 0.03 ug/kg, respectively) and the calculated cumulative exposure levels for 2,4,5-T in Situations 1, 2, and 3 and TCDD in Situation 1 (see Table 34) do not constitute an ample margin of safety. The Working Group therefore recommends issuance of a rebuttable presumption against pesticide products containing 2,4,5-T pursuant to 40 CFR Section 162.11(a)(3)(ii)(B).

IV. STUDIES RELATING TO POSSIBLE ADVERSE EFFECTS

This section addresses other types of adverse effects of 2,4,5-T for which the Working Group has determined that insufficient evidence exists to initiate a rebuttable presumption. The Agency solicits comments from registrants and other interested parties on the evidence listed below, and requests submission of any additional studies or relevant information on 2,4,5-T and/or TCDD relative to these potential adverse effects.

A. Mutagenicity

Section 162.11(a)(3)(ii)(A) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) induce mutagenic effects, as determined by multitest evidence.

(1) 2,4,5-T

(a) Positive Study

Majumdar and Golia (178) fed male *Drosophila melanogaster* either 250 or 1,000 ppm dioxin free 2,4,5-T (obtained from Eastman Kodak) for 15 days. They were then mated to sets of virgin females to generate three 4-day broods of offspring. F_1 flies were allowed to mate, and F_2 flies were scored for X-linked recessive lethals. No differences among

broods were noted, and data from all broods were pooled. The percent lethals in controls, 250 and 1,000 ppm groups were dose-related and were 0.05, 0.26, and 0.66%, respectively. The control vs. 1,000 ppm lethal rates were significantly different from one another ($p < 0.01$). Ethyl methane sulfonate (250 ppm) was included as a positive control; it yielded 13.70% lethals. The total number of flies in each experimental group was no larger than 2,000.

(b) Negative Studies

The mutagenicity of 2,4,5-T was evaluated by Ercegovich et al. (148), employing the procedure of Ames, using five strains of Salmonella typhimurium without activation. They concluded that 2,4,5-T is not mutagenic.

Fujita et al. (149) reported chromosomal abnormalities in in vitro cytogenetic studies of human lymphocytes exposed to 10^{-7} to 10^{-4} M of 2,4,5-T, which contained 0.09 ppm TCDD. Breaks, deletions, and rings were observed. Chromatid breaks increased with increasing concentrations of 2,4,5-T. It was not possible to distinguish whether this was a toxic effect or a potential genetic effect (150).

Majumdar and Hall (169) reported on the cytogenetic effects of 2,4,5-T[18/] on *in vivo* bone-marrow cells of Mongolian gerbils. The animals were injected with total amounts of 2,4,5-T at the rate of 50, 150, 250, 350, or 500 mg/kg body weight over the 5-day period of the study. Increasing numbers of chromatic gaps, breaks, and fragments were observed at 250, 350, and 500 mg/kg doses. No exchange figures or isochromosome gaps or breaks were observed. This is not a definitive experiment for indicating the potential of 2,4,5-T for causing heritable chromosome damage (170). Toxicity effects of the chemical could give similar results (170).

Davring and Hultgren (171) reported on an *in vivo* study on the cytogenetic effects on bone-marrow cells of *Mus musculus* (male mice) induced by a Swedish commercial 2,4,5-T ester formulation[19/] and its components. The study showed that 2,4,5-T commercial products can affect chromosomal and reproductive mechanisms. Two different strains of mice were used with similar results for both. These results correlated with effects seen in Drosophila. The authors stated that chromatid

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18/ The 2,4,5-T used in this study was purchased from Eastman Kodak Company, Rochester, N.Y., and contained no measurable amount of TCDD. The authors do not indicate the limit of sensitivity.

19/ The concentration of TCDD was guaranteed to be less than 0.1 ppm in the product.

inter- or intraexchanges were never observed. This study was not carried out sufficiently for the demonstration of chromosomal effects such as rearrangements in future generations of somatic cells (170).

Davring and Sunner (172) demonstrated cytogenetic effects of a Swedish commercial 2,4,5-T formulation[20/] on oogenesis and early embryogenesis in Drosophila melanogaster. A 50% decrease in fertility for the flies was determined to be 250 ppm. This level is 40 to 60 times less than field use concentration levels. Reproductive and chromosomal effects were observed.

(2) TCDD

(a) Positive Studies

Hussain et al. (24) evaluated the mutagenic activity of TCDD (99% pure) on three different microbial test systems. In the first study, TCDD significantly increased the incidence of reverse mutations in Escherchia coli Sd-4 administered 2 ug/ml TCDD from streptomycin dependence to streptomycin independence. This was the only dose at which mutations were clearly observed. No details of the experimental

20/ TCDD concentration was less than 0.1 ppm in this formulation which was tested at practical field use concentrations or lower.

protocol were given, and statistical methods were apparently not employed in assessing the data.

The second test by Hussain et al. (24) studied reverse mutation from histidine dependence to histidine independence in Salmonella typhimurium (Strains TA 1532 and TA 1530). TCDD was positive in TA 1532 but negative in TA 1530. This indicated that TCDD acts as a frameshift mutagen. ICR-170 was used as a positive control in the test with TA 1532. No positive or negative controls were tested in TA 1530.

In the third test Hussain et al. (24) observed slight prophage induction in E. coli K-39. However, data from this test were difficult to evaluate because the solvent used, dimethyl sulfoxide, causes cellular effects.

A preliminary report on the chromosomal analysis of hospital patients exposed to TCDD in the accident at the Seveso, Italy, factory was presented at the Department of Health, Education, and Welfare meeting on October 12, 1976 (152). An increased number of chromosomal lesions (gaps, chromatidic and chromosomal breaks, and rearrangements) were observed in somatic cells of the 2- to 28-year-old males and females tested. Cytogenetic studies of tissues from therapeutic abortions performed on women who were exposed to TCDD during the accident indicated that there was chromosomal

damage to cells in maternal peripheral blood, and placental and fetal tissues. These preliminary results were based on a small number of samples, and no specific data are available at this time (150).

(b) Negative Studies

Khera and Ruddick (6) conducted dominant lethal tests in which male Wistar rats received TCDD at dosages of 4 and 8 ug/kg per day. The studies indicated that no dominant lethal mutations arose during the 35 days after treatment. The period examined corresponded to postmeiotic stages of spermatogenesis.

A cytogenetic screening study of the effects of TCDD on bone marrow cells of male Osborne-Mendel rats was performed by the Food and Drug Administration (119). Two separate experiments were performed. The first was a multiple dose test in which 10 ug TCDD/kg per day was administered by intubation for 5 consecutive days. In the second test, single doses of 5, 10, and 15 ug TCDD/kg were administered intraperitoneally and 20 ug/kg (the highest dose) was administered orally. There was no evidence from these studies to indicate that TCDD produced cytogenetic damage in the bone marrow of these male rats. Toxicity, which was indicated by a slight weight loss, was noted in rats that received a single dose of 15 or 20 ug/kg (the highest dose levels).

Green (119) conducted a short-term investigation of several dioxins, using male Osborne-Mendel rats, to determine what potential these substances had to produce cytogenetic damage in rat bone-marrow. In one study all of the dioxins were tested by being intubated in the rats for five consecutive days at 10 ug/kg per day. A second study involved TCDD alone administered orally at 20 ug/kg and intraperitoneally at 5, 10, and 15 ug/kg. The author found no evidence that any of the substances tested produced cytogenetic damage in the bone marrow of male rats.

In conclusion, although Hussain et al. (24) have demonstrated that TCDD does appear to act as a point (gene) mutagen, the evidence is weak for heritable genetic effects since the level of mutagenic testing is meager and there were some major deficiences in some tests. However, the study by Hussain et al. does not fulfill the criterion of multitest evidence as prescribed in 40 CFR 162.11. Although TCDD does appear to have the potential to act as a chromosomal mutagen from the in vivo cytogenetic studies (152), specific data are not yet available from the Seveso accident.

(3) Chromosomal Damage

The Working Group also wishes to call attention to three studies [previously discussed in Sections III.B.(2)(b), IV.(1), and IV.(2)(b)], which indicate that 2,4,5-T and/or

TCDD may cause chromosomal damage. Fujita et al. (149) reported chromosomal abnormalities in in vitro tests on human lymphocytes exposed to 2,4,5-T; abnormalities included breaks, deletions, and rings. Yefimenko (167) reported damage to bone-marrow cell chromosomes (including breaks, true aberrations, or rearrangements) in in vivo tests on rat gonadal and somatic tissue exposed to butyl ester 2,4,5-T. The preliminary HEW report (152) on the Seveso incident indicated an increased number of chromosomal lesions (gaps, chromatic breaks, and rearrangements) in somatic cells of 2- to 28-year-old humans exposed to TCDD.

The Working Group concludes that there is a data gap on mutagenic effects and that further evidence and testing is needed on the mutagenicity of 2,4,5-T and TCDD. The Working Group would like to evaluate more detailed and specific information as it becomes available from the Seveso accident. Relevant information or studies on the mutagenetic effects of 2,4,5-T and/or TCDD should be submitted to the Agency, and the option for re-evaluating their mutagenic properties must be left open should more conclusive evidence become available.

B. Toxicity to Humans: TCDD

(1) Chloracne

A number of researchers have reported illness ascribed to TCDD (90, 93, 95, 153). Most of these toxic effects have

occurred in chemical plant workers after accidental exposure to the dioxin. While a number of ill effects have been reported, the most widely known is chloracne.

Chloracne is a severe skin disease resulting from exposure to highly chlorinated dibenzo dioxins. It is a disease of the follicular and sebaceous glands. Its symptoms and signs include skin lesions, follicular hyperkeratosis, and the formation of large sebaceous cysts, inflammed tubercles, and pustules. In addition to these symptoms, chloracne is often accompanied by a brownish keratinization of the skin, cystitis, pyelonephritis, depression, hirsutism, fatigue, neurological disturbances, raised cholesterol levels, liver damage, and psychological manifestations (10, 15, 16, 154, 155, 156, 157). Several researchers have observed that chloracne is not only irritating and persistent but also very difficult to cure. It is one of the most frequently contracted forms of occupational dermatitis, occurring primarily in chemical plant employees engaged in the production of 2,4,5-T and 2,4,5-TCP (16, 95, 155, 156, 158).

The first report on a toxic material being the causative agent for an occupational skin disease appears to have been by Dr. Karl Herxheimer in 1899. Dr. Herxheimer diagnosed the cause of dermatological problems in a German

factory worker as exposure to chlorine ions in the production of caustic potash (159). It is from this early diagnosis that we get the name chloracne. During the early 1950's there were a series of industrial accidents in Germany resulting in an outbreak of chloracne in the employees of chemical plants manufacturing 2,4,5-T and 2,4,5-TCP. The symptoms of the employees of one of these factories in Hamburg, Germany, were extensively investigated by Kimming and Schulz (16). These researchers, using the rabbit ear test, proved that the cause of the chloracne was a contaminant found in crude 2,4,5-TCP and not the formulated 2,4,5-TCP. Later on, Bauer et al. (15) conclusively identified TCDD as the causative agent of chloracne.

(2) Porphyria cutanea tarda and δ-Aminolevulinic Acid Synthetase

Porphyria cutanea tarda (PCT), a form of hepatic porphyria, is another disease caused by exposure to TCDD and often accompanies chloracne. PCT occurs primarily in industrial workers associated with the manufacture of 2,4,5-T (93, 94, 160).

The symptoms of porphyria cutanea tarda, a defect in hepatic metabolism of porphyrins, are fragility of the skin, photosensitivity of the skin, hyperpigmentation, over-production of porphyrins, hirsutism, and neurological

and intestinal disorders (94, 160). It is also characterized biochemically by an increase in the activity of the mitochondrial enzyme δ-aminolevulinic acid (ALA) synthetase, which is the first and rate-limiting enzyme in heme biosynthesis (160). TCDD was thought to be a potent inducer of ALA activity in chick embryo liver (115). Goldstein et al. (161) reported that TCDD was found to induce ALA synthetase and hepatic prophyria in mice. These researchers stated that at that time [1973] TCDD was the most potent porphyrogenic chemical known. Poland and Kende 1976 (4) found that the duration of ALA induction from TCDD exposure is prolonged, most likely due to the long biological half-life of TCDD. These researchers also found that ALA synthetase inducers have halogen atoms occupying at least three of the four lateral ring positions (positions 2, 3, 7, and 8), and that there is at least one free, nonhalogenated ring position. TCDD fulfills all of these requirements.

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