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Research and Development

RISK ASSESSMENT ON
(2,4,5-TRICHLOROPHENOXY) ACETIC ACID (2,4,5-T)
(2,4,5-TRICHLOROPHENOXY) PROPIONIC ACID
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

Prepared for

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Prepared by

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Carcinogen Assessment Group

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16. ABSTRACT

Carcinogenic responses have been induced in mice and rats at low doses of TCDD. TCDD has been shown to be a cancer promoter. These results, together with the strongly suggestive evidence in epidemiology studies, constitute substantial evidence that TCDD is likely to be a human carcinogen. It appears that TCDD is a more potent carcinogen than aflatoxin B_1 which is one of the most potent carcinogens known. The levels of TCDD (contained as an unavoidable contaminant of the 2,4,5-T) used in the 2,4,5-T studies apparently were too small to produce an observable response in those experiments. The lack of a statistically significant tumor incidence in most of the studies on the 2,4,5-T product may be attributed to the very low levels of TCDD in the product relative to the levels at which it produced carcinogenic effects in rats and mice, as well as to deficiencies of those studies. However, since TCDD is a carcinogen, any product containing TCDD, including 2,4,5-T and silvex, can be considered to pose a human carcinogenic hazard. Furthermore, a rat study on specially purified 2,4,5-T provides highly suggestive evidence that essentially pure 2,4,5-T may be a human carcinogen. Quantitative assessments have been calculated for the carcinogenic risk posed to humans.

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CORRECTIONS TO CARCINOGEN ASSESSMENT GROUP'S RISK ASSESSMENT
ON 2,4,5-T, SILVEX, AND TCDD
(Dated September 12, 1980)

Page	Line	Present	Should Be
104	1	ae	are
106	18-19	that apply 2,4,5-T	(omit)
106	18	the applicators	the 2,4,5-T applicators
106	21	Pg. 13	Pg. 14
109	18	exposures	exposure
109	18	4.7×10^{-4}	4.5×10^{-4}
110	9	high consumer group	local population
110	19	as high as or	(omit)
110	last	4.7×10^{-4}	4.5×10^{-4}
111	4	were	is
115			delete footnote b
116	3	Females	Females[a]
116			Delete footnote and replace with: [a]Subcutaneous combined fibroma or fibrosarcoma not significant
120	Table 49	Revised Table attached	
130	Table 59	Revised Table attached	
131	7	8.4×10^{5}	8.4×10^{-5}
132	11	210^{-4}	$< 10^{-4}$
133	10	4.8×10^{-3}	5.2×10^{-3}
134	6	Local population*	(omit *)
135	9	pg/kg/bw/day	pg/kg bw/day
137	9	4.7×10^{-4}	4.5×10^{-4}

TABLE 49. CURVE FIT OF THE MULTISTAGE MODEL PARAMETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOGIST
LINEAR PARAMETER q_1, MAXIMIZED TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....Kociba - Dow
Sex-species.....Male rat
Weight (w_a).....600 gm
Tumor sites (one or more)....Tongue - squamous cell carcinomas
Nasal turbinates/hard palate - stratified squamous cell carcinoma

Pathologist - Kociba

Exposure level (mg/kg/day)	0	1×10^{-6}	1×10^{-5}	1×10^{-4}
+r/n	0/76	2/49	1/49	3/42

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	1.40×10^{-2}	1.10×10^{3}	0	5.86×10^{10}	3.01×10^{3}	3.34 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P > 0.01$) = 3.01×10^3

$q_h^* = q_1^* (70/w_a)^{1/3} = 1.47 \times 10^4$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 59. HUMAN SLOPE ESTIMATES

Compound	Species	Study	Sex	Pathologist	Human Slope Estimate q_h^*
TCDD	Rat	Dow	Male	Kociba	1.47×10^4
				Squire	1.73×10^4
			Female	Kociba	2.52×10^5
				Squire	$4.25 \times 10^{5*}$
		NCI	Male	NCI - Reviewed	2.43×10^4
			Female	NCI - Reviewed	3.28×10^4
	Mice	NCI	Male	NCI - Reviewed	1.33×10^5
			Female	NCI - Reviewed	4.56×10^4
2,4,5-T	Rat	Dow	Male	Kociba	1.65×10^{-2}
				Squire	$1.82 \times 10^{-2*}$

*Values used in risk analysis

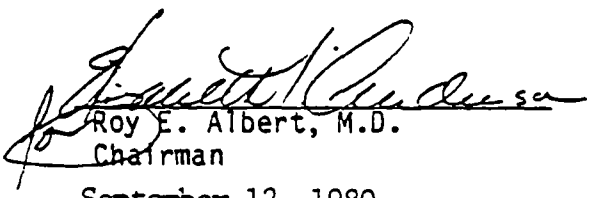
THE CARCINOGEN ASSESSMENT GROUP'S

RISK ASSESSMENT ON

(2,4,5-TRICHLOROPHENOXY)ACETIC ACID (2,4,5-T)

(2,4,5-TRICHLOROPHENOXY)PROPIONIC ACID (SILVEX)

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)



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The CAG reports are prepared for internal Agency use in response to EPA's regulatory office needs. They range from brief chemical profiles to very extensive evaluations, depending upon the nature of a request. The reports are used by the regulatory offices for regulatory decision making as appropriate. The reports are revised and edited based on regulatory office needs and the availability of resources.

This document was prepared at the request of the EPA Office of the General Counsel.

CONTENTS

SUMMARY AND CONCLUSIONS

QUALITATIVE RISK ASSESSMENT

(2,4,5-Trichlorophenoxy)Acetic Acid (2,4,5-T)

(2,4,5-Trichlorophenoxy)acetic acid, widely known as 2,4,5-T is used as a vegetation growth regulator and herbicide. "Agent Orange," a defoliant used extensively by the U.S. Army in Vietnam, is a mixture of equal amounts of 2,4,5-T and (2,4-dichlorophenoxy)acetic acid. In 1970, amid growing concern about the teratogenic effects of 2,4,5-T, the EPA cancelled the registration of the compound for uses "around the home, recreation areas, and similar sites" and "in crops intended for human consumption." Before some uses were suspended in 1979, it was used primarily to clear vegetation along powerlines, highways, pipelines, and railroad rights-of-way, and on range, pasture, and forestlands.

The commercial preparation of 2,4,5-T contains 2,3,7,8-tetrachlorodibenzo -p-dioxin (TCDD) as an unavoidable impurity present at a concentration of approximately 0.05 ppm. TCDD is considered extremely toxic.

2,4,5-T is readily absorbed by several mammalian species, including man, and is excreted unchanged - mostly in urine.

The available information about the mutagenic activity of 2,4,5-T is considered to be limited. 2,4,5-T is indicated to be a weak mutagen in Drosophila and, under acidic conditions, showed mutagenic effects in Saccharomyces cerevisiae.

Tests for the chronic carcinogenicity of 2,4,5-T were performed by several investigators. Two studies were carried out with Sprague-Dawley rats, one by the Dow Chemical Company (Kociba et al. 1979) and one by F. Leuschner (1979), Laboratorium fur Pharmakologie und Toxikologie, Hamburg, Germany. The Dow study showed an increased incidence of carcinoma of the tongue in male rats dosed with

specially purified 2,4,5-T at 30 mg/kg/day. This incidence as reported by the authors of the study, is marginally statistically significant ($P = 0.063$) when compared to controls. In addition, there was a significant dose-related linear trend by the Cochran-Armitage test. When compared to historical controls, the incidence of this tumor, as reported by the authors, is statistically significant ($P < 0.001$); however, when the tongue tissues from this study were reexamined by Dr. Squire, he found one additional tongue carcinoma in male rats treated at the high dose with 2,4,5-T which increased the statistical significance to $P = 0.025$. In a recently completed study by F. Leuschner, an increased incidence of interstitial cell tumors of the testes was observed when compared with matched controls. However, this increase is not statistically significant when compared to historical controls. The results of the Kociba et al. study provide highly suggestive evidence of the carcinogenicity of essentially pure 2,4,5-T.

In mice, two studies by Muranyi-Kovacs et al. (1976, 1977) and two studies by Innes et al. (1969) (Bionetics Laboratories 1968) have not provided positive evidence of oncogenic effects of 2,4,5-T. However, several deficiencies in these studies make them inadequate to assess the lack of oncogenicity of 2,4,5-T.

In summary, the Dow study in rats provides highly suggestive evidence of the carcinogenicity of 2,4,5-T, while the Leuschner study showed only equivocal results. The mouse studies were too insensitive to be considered valid negative studies.

(2,4,5-Trichlorophenoxy)Propionic Acid (Silvex)

Silvex, like 2,4,5-T, contains the highly toxic TCDD. Uses of silvex are similar to those of 2,4,5-T. Chronic carcinogenicity studies have been performed on mice and rats and a 2-year study has been conducted on dogs. Innes

et al. (1969) (Bionetics Laboratories 1968) conducted two studies using mice, one oral and the other subcutaneous. These studies were found to be inadequate to assess the carcinogenicity of silvex.

Dow Chemical Company performed two feeding studies, a 2-year feeding study on rats and a two year feeding study on dogs which were summarized by Mullison (1966) and Gehring and Betso (1978). These have been found to be inadequate to rule out the carcinogenicity of silvex.

2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD)

Probably one of the most toxic chemicals known to man is 2,3,7,8-tetrachlorodibenzo-p-dioxin. The major source of its environmental contamination is from the pesticidal uses of 2,4,5-T, 2,4,5-trichlorophenol, and silvex.

In small amounts, TCDD is a potent inducer of arylhydrocarbon hydroxylase in mammals. This is a complex enzyme system that consists of epoxidase, epoxidehydratase, and glutathione transferase. The enzyme epoxidase is known to mediate the formation of epoxides, which are potentially active carcinogenic metabolites. TCDD can be metabolized in mammalian species via the epoxide to dihydodiol and further conjugates with glutathione. Persistent residues of TCDD were found in liver and fat in a 2-year feeding study in rats. Significant covalent binding of TCDD to protein has been demonstrated by two investigators. Covalent binding of TCDD with DNA is less significant in liver cells.

Currently available studies on the mutagenicity of TCDD are inconclusive. Two bacterial systems, Escherichia coli and Salmonella typhimurium (without metabolic activation), exhibited positive mutagenic activity. However, in another study of Salmonella typhimurium (with and without metabolic activation), the results were negative.

There are several cancer bioassay studies of TCDD: 1) a Dow Chemical Company (Kociba et al. 1978) study in male and female Sprague-Dawley (Sprague substrain) rats; 2) the Van Miller et al. (1977) study in male Sprague-Dawley rats; 3) the Toth et al. (1979) study in Swiss mice; 4) the National Cancer Institute (1980a, b) studies in rats and mice; 5) the Pitot et al. (1980) promotion study in rats; and 6) the Kouri et al. (1978) cocarcinogenicity study in mice.

The study by the Dow Chemical Company of male and female Sprague-Dawley rats fed TCDD in doses of 22 ppt, 210 ppt, and 2200 ppt revealed a highly statistically significant excess incidence of hepatocellular carcinomas in female rats at the highest dose level and hepatocellular carcinomas and hepatocellular hyperplastic nodules in female rats at the middle dose level, as compared to the controls. In addition, there was a significant increase in carcinomas of the hard palate/nasal turbinates in both high dose males and females, of the tongue in males, and of the lung in females. The Van Miller et al. study also showed some evidence of a carcinogenic response in the liver and lungs of male Sprague-Dawley rats at dosages of 1000 and 5000 ppt, even though the study used a relatively small number of animals. The Toth et al. study provides suggestive evidence that TCDD induced an increased incidence of liver tumors in male mice (females were not tested) receiving 0.7 ug/kg/week by gavage.

In the National Cancer Institute rat study (1980a), male and female Osborne-Mendel rats were administered TCDD by gavage at three dose levels 0.01, 0.05, and 0.5 ug/kg/week. TCDD induced statistically significant increases of hepatocellular carcinomas, subcutaneous fibrosarcomas, and adrenal cortical adenomas in high dose female rats. TCDD also induced significant increases in thyroid tumors at low, middle, and high doses in male rats.

In a companion mouse study by the National Cancer Institute (1980a), male and female B6C3F1 mice were given TCDD by gavage at dose levels of 0.01, 0.05, and 0.5 ug/kg/week for males and 0.04, 0.2, and 2.0 ug/kg/week for females. TCDD induced statistically significant increased incidences of hepatocellular carcinomas in the high dose males and females, and thyroid tumors, subcutaneous fibrosarcomas, and histiocytic lymphomas in females.

In a study by Pitot et al. (1980), TCDD has been shown to be a potent liver cancer promoter. In a study by Kouri et al. (1978), TCDD has been shown to be a cocarcinogen.

Epidemiologic Studies

Several epidemiologic studies have been conducted which are relevant to the assessment of the carcinogenicity of 2,4,5-T, silvex, and TCDD. Two Swedish epidemiological case-control studies (Hardell and Sandstrom 1979, Erikson et al. 1979) reported a very strong association between soft tissue sarcomas and occupational exposure to phenoxyacetic acid herbicides and/or chlorophenols. These studies indicated approximately five to sevenfold increases in the risk of developing soft tissue sarcomas among people exposed to phenoxyacetic acids only in comparison to people not exposed to these chemicals. Another Swedish case-control study (Hardell et al. 1980) provides suggestive evidence of an increased risk of developing lymphomas resulting from occupational exposure to phenoxyacetic acids.

Two cohort studies, one by Axelson et al. (1980) and the other by Thiess and Frentzel-Beyme (1977) provide suggestive evidence that phenoxyacetic acids and/or TCDD increases the risk of stomach cancer in humans.

Four other cohort studies by Ott et al. (1980), Riihimaki et al. (1978), Zack and Suskind (1980), and Cook et al. (1980) did not indicate an increased

risk of stomach cancer, but three of these studies were of relatively low statistical power, and the fourth (Riihimaki et al. 1977) has certain inconsistencies requiring clarification.

In summary, carcinogenic responses have been induced in mice and rats at very low doses of TCDD. In addition, TCDD has been shown to be a potent cancer promoter. These results, together with the strongly suggestive evidence in epidemiologic studies, constitute substantial evidence that TCDD is likely to be a human carcinogen. In addition, on the basis of the Dow study on TCDD, it appears that TCDD is a more potent carcinogen than aflatoxin B_1 which is one of the most potent carcinogens known. The levels of TCDD (contained as a contaminant of the 2,4,5-T) used in the 2,4,5-T studies apparently were too small to produce an observable response in those experiments. The lack of a statistically significant tumor incidence in most of the studies on the 2,4,5-T product may be attributed both to the very low levels of TCDD in the product relative to the levels at which it produces observable carcinogenic effects in rats and mice, as well as to the deficiencies of those studies. However, since TCDD is a carcinogen, any product containing TCDD, including 2,4,5-T and silvex, can be considered to pose a human carcinogenic hazard. In addition, a rat study on specially purified 2,4,5-T provides highly suggestive evidence that essentially pure 2,4,5-T may be a human carcinogen.

QUANTITATIVE RISK ASSESSMENT OF 2,4,5-T, SILVEX AND TCDD

A quantitative assessment has been calculated for the carcinogenic risk posed to humans by the use of the herbicides 2,4,5-T and silvex. While there is no evidence for carcinogenicity of silvex, the evidence for 2,4,5-T is highly suggestive, and that for the contaminant TCDD is substantial. Furthermore, TCDD is highly carcinogenic to animals.

The assessment of risk from TCDD exposure covers only the herbicide applicators and dietary exposure to beef, milk, deer, and elk. For unprotected workers, the upper limits of lifetime risk of induced cancers are in many cases as high as or in the 10^{-3} range. For the general population exposed to beef contaminated with TCDD, the upper limit of risk for the estimated exposure is 2.4×10^{-6}. For local populations consuming only beef which is contaminated with TCDD, the risk is much greater, as high as 1.9×10^{-4} for the estimated exposure. For local populations consuming only milk and other dairy products which are contaminated with TCDD, the risk is 4.7×10^{-4}. For deer and elk meat contaminated with TCDD, risks to the local population are no greater than 10^{-4} for 12 meals a year.

The upper limit of dietary risk associated with estimated exposures to 2,4,5-T in contaminated rice and milk were in the 10^{-7} range for a high consumer eating only contaminated rice or an average consumer drinking only contaminated milk.

QUALITATIVE RISK ASSESSMENT

I. INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an extremely toxic contaminant that forms when tetrachlorobenzene is hydrolyzed in an alkaline ethylene glycol solution to produce 2,4,5-trichlorophenol. The amount of TCDD produced increases with an increase in the temperature of the reaction. The 2,4,5-trichlorophenol is used as an intermediary in the production of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) and (2,4,5-trichlorophenoxy)propionic acid (silvex). Therefore, the TCDD contaminates both products to the same extent (see Figure 1, below).

TCDD can also occur in other chlorinated phenols and in the chemicals synthesized from them. TCDD does not occur naturally in the environment, but exists only as a contaminant of other chemicals.

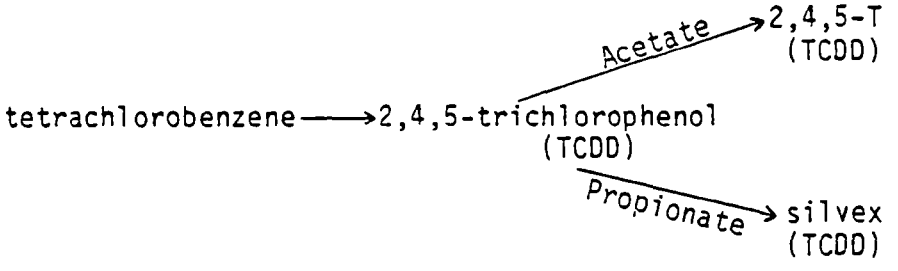
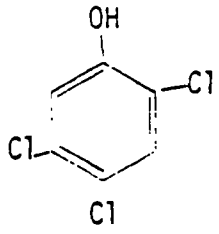


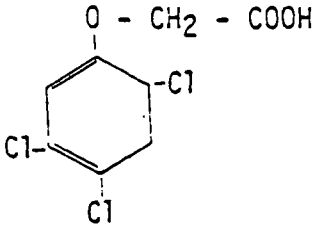
Figure 1. Formation of TCDD, 2,4,5-trichlorophenol, 2,4,5-T, and silvex.

In the 1960s, the TCDD content in commercial 2,4,5-T and 2,4,5-trichlorophenol ranged from 5 to 50 ppm. By the early '70s, the manufacturers had set a limit of 0.1 ppm TCDD contamination in their products.

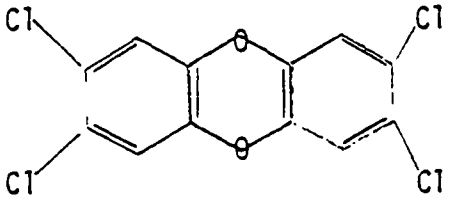
The structure of the four compounds is shown in Figure 2 below.



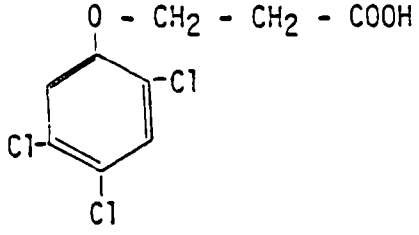
2,4,5-trichlorophenol
(2,4,5-TCP)



(2,4,5-trichlorophenoxy)acetic acid
(2,4,5-T)



2,3,7,8-tetrachlorodibenzo-p-dioxin
(TCDD)



(2,4,5-trichlorophenoxy)propionic acid
(silvex)

Figure 2. Structure of TCDD and TCDD-containing compounds.

2,4,5-T is used as a growth regulator and herbicide. The herbicide "Agent Orange," used extensively by the U.S. Army as a defoliant in Vietnam, is a mixture of equal amounts of 2,4,5-T and (2,4-dichlorophenoxy)acetic acid. In 1970, amid growing concern about the teratogenic effects of 2,4,5-T, the EPA cancelled registration of the compound for uses "around the home, recreation areas, and similar sites" and "on crops intended for human consumption." Until EPA suspended certain uses in 1979, it was used primarily to clear vegetation along powerlines, highways, pipelines, and railroad rights-of-way, and on range, pasture, and forestlands.

II. METABOLISM

METABOLISM OF (2,4,5-TRICHLOROPHENOXY)ACETIC ACID (2,4,5-T)

The metabolic fate of 98% pure 2,4,5-T was studied in beagle dogs and adult Sprague-Dawley rats following a single oral dose of the chemical (Piper et al. 1973). The absorption of 2,4,5-T appeared to follow first order kinetics in rats and dogs. The rate at which the compounds cleared from plasma was also of the first order in rats, but in dogs, the clearance rate was much more complex than first order.

The t1/2 values for clearance of ^{14}C-activity from the plasma of rats given doses of 5, 50, 100, or 200 mg/kg were 4.7, 4.2, 19.4, and 25.2 hours, respectively. The volume of distribution also apparently increased with dose. In dogs given 5 mg/kg, the t1/2 values for clearance from plasma and elimination from the body were 77.0 and 86.6 hours, respectively.

Essentially all of the 2,4,5-T was excreted unchanged in the rats' urine, except for a small amount of one unidentified metabolite that was detected only in rats administered the two highest doses. Urinary excretion accounted for most of the 2,4,5-T eliminated from the body in rat ; little was found in the feces.

In dogs, a greater percentage of 2,4,5-T was excreted in the feces than in the urine. Three unidentified metabolites of 2,4,5-T were detected in the urine, but there may have been fecal contamination, so the source is equivocal. The slower elimination of 2,4,5-T in the body of the dog may account for its greater metabolic alteration. The authors suggest that the kidney possesses a saturable active transport system for 2,4,5-T and this transport system has a greater capacity in adult rats than in dogs. The longer half-life of elimination and the metabolic degradation of 2,4,5-T in dogs may explain why

2,4,5-T is more tc ic to dogs than to rats.

Five male human volunteers ingested a single 5 mg/kg dose of 99% pure 2,4,5-T containing 0.05 ppm TCDD (Gehring et al. 1973). The plasma concentration of 2,4,5-T increased rapidly and peaked at 57 ug/ml following 7 hours of administration. The subsequent clearance rates from the plasma and body were of first order, situated numerically between the rates for dogs and for rats. The 2,4,5-T was actively secreted in the urine. It was concluded that 2,4,5-T is eliminated fairly unchanged from the human body. The volume distribution in humans was smaller than for test animals. In humans, 65% of the compound remaining after 24 hours was present in plasma, and 99% of this was reversibly bound to protein.

In conclusion, 2,4,5-T is readily absorbed by several mammalian species including man, and excreted mostly in the urine.

METABOLISM AND STORAGE OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

In a 1976 study by Rose et al., Sprague-Dawley rats were given either a single oral dose of 1.0 ug ^{14}C-TCDD/kg (98% pure with 2% trichlorodibenzo-p-dioxin) or repeated oral doses of 0.01, 0.1, or 1.0 ug ^{14}C-TCDD/kg/day, 5 days per week, for 7 weeks.

The authors monitored the fate of ^{14}C-TCDD in rats after single oral administration and found that, on the average, 83% of the dose was absorbed. Twenty-two days after the single oral dose, concentrations of ^{14}C-activity were retained mainly in the liver (1.26% of dose) and fat (1.25% of dose). The half-life of ^{14}C following a single oral dose was 31 $\pm$ 6 days, which followed first order kinetics. Most of the ^{14}C-activity was detected in feces and not in urine or expired air, which indicates that TCDD and/or its metabolites are eliminated via the bile.

The authors also monitored the fate of the ^{14}C-TCDD ingested repeatedly. Following the administration of all doses of ^{14}C-TCDD, the average dose absorbed was 86%. The rats were killed at 1, 3, or 7 weeks, and their liver, fat, kidney, thymus, and spleen tissues were examined for ^{14}C-activity. Activity was primarily localized in the liver and fat, with radioactivity in the liver being five times greater than in fat. The accumulation of the isotope in both types of tissue followed first order kinetics. With continuous administration, the concentrations of ^{14}C-radioactivity in both tissues approached plateau levels by 7 weeks (73.8% of steady state values). By 13 weeks, 93% of the steady state level had been reached; the rate of accumulation of radioactivity was independent of the dose level administered over the dose range of 0.01 to 1.0 ug TCDD/kg/day.

The half-life of elimination of ^{14}C-activity in rats was 23.7 days. The ^{14}C-TCDD radioactivity was excreted primarily in the feces, with some of it in an altered chemical form, presumably having been excreted from the liver via bile. Significant amounts of ^{14}C-TCDD were also found in urine, particularly in female rats. Female rats were given 0.001, 0.01, and 0.1 ug TCDD/kg of body weight for 2 years (Kociba et al. 1978). After being killed, the liver and fat tissues were analyzed for TCDD content. The chemical analysis of liver and fat are shown in the Table 1 below. These results reveal the dose-dependent accumulation of TCDD after long-term exposure.

TABLE 1. CONCENTRATIONS OF TCDD IN RAT LIVER AND FAT AFTER 2 YEARS OF FEEDING

Dose	Concentration in liver[a]	Concentrations in fat[a]
0.001 ug/kg	540	540
0.01 ug/kg	5,100	1,700
0.1 ug/kg	24,000	8,100

[a]parts per trillion

ARYL HYDROCARBON HYDROXYLASE (AHH) INDUCTION STUDIES WITH TCDD

TCDD causes toxic effects, which are discussed in Section V of this document. The biochemical lesions underlying the observed toxicologic effects of TCDD are not known, but certain enzyme systems have been shown to change when animals are exposed to non-lethal doses of TCDD (Hook 1975). In particular, hepatic microsomal mixed-function oxidases seem to be highly responsive to TCDD.

AHH is one of the microsomal mixed-function oxidase enzyme systems responsible for the oxidative metabolism of many exogenous and endogenous compounds, including many polycyclic aromatic hydrocarbons (Poland and Glover 1973, Kouri 1976). The metabolic oxidation of these compounds proceeds via transient chemically reactive intermediates, including epoxides (Kouri 1976).

The AHH enzyme system is induced by a wide variety of drugs and polycyclic aromatic hydrocarbons, including the steroid hormones, benzo(a)pyrene and 3-methylcholanthrene, as well as TCDD and compounds that structurally resemble TCDD, i.e., polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzofuran, 3,4,3',4'-tetrachloroazoxybenzene, and 3,4,3',4'-tetrachloroazobenzene (Poland and Glover 1976[b], Goldstein et al. 1977, Kouri et al. 1973).

Kouri et al. (1973) correlated induction of AHH by 3-methylcholanthrene

(3-MC) in 14 mouse strains with a high carcinogenic index, a measure of sensitivity to chemical carcinogens which are metabolically activated to a carcinogenic intermediate, and found that the AHH system plays a role in this activation.

TCDD is reported to produce effects on microsomal mixed-function oxidases that are very similar to those produced by 3-MC (Poland and Glover 1974). Many compounds have been shown to induce certain mixed-function oxidases, but enzyme-inductive plus enzyme-suppressive effects are peculiar to inducers like 3-MC and TCDD. The mechanisms involved in regulation of the mixed-function oxidases vary with the organ and species (Hook 1975). The potency of TCDD in inducing hepatic AHH is 3×10^4 that of 3-MC (Poland and Glover 1974), and is 40 to 60 times that of 3-MC in inducing AHH activity in cultured human lymphocytes (Kouri and Ratrie 1974).

There are genetic differences in AHH inducibility in humans and in mice (Poland and Glover 1976[b], Kouri and Ratrie 1974). Poland and Glover (1976[b]) inferred from mouse data that the hepatic cytosol species that binds TCDD is the receptor for the induction of hepatic AHH activity, and the mutation in non-responsive mice results in an altered receptor with a diminished affinity for inducing compounds.

In conclusion, TCDD is a potent inducer of arylhydrocarbon hydroxylase in mammalian species. This is a complex enzyme system which consists of epoxidase, epoxidehydratase, and glutathione transferase. The enzyme epoxidase is known to mediate the formation of epoxides, which are potentially reactive carcinogenic metabolites. TCDD undergoes metabolic transformation in mammalian species; however, its persistent residues were found in liver and fat after 2-year feeding studies in rats.

COVALENT BINDING OF TCDD WITH MACROMOLECULES

There are two relevant studies that deal with the interaction of 2,3,7,8-tetrachlorodibenzo-p-dioxin with macromolecules. In the first study by Guenthner et al. (1979), covalent binding of TCDD metabolites to cellular macromolecules was measured *in vitro* after incubation of tritiated TCDD with methylcholanthrene-induced B6C3F1 mouse microsomes, NADPH, and deproteinized salmon DNA. The ratio of amount of DNA to the amount of protein in the reaction vessel was 4:1. After incubation, the DNA was reisolated and treated with DNase, phosphodiesterase, and alkaline phosphatase. TCDD metabolite-nucleoside adducts were isolated by sephadex LH_{20} column chromatography. The radioactivity equivalent to TCDD that binds with DNA was 0.074 p mole/mg. When DNA was incubated with proteinase before being applied to the sephadex column, more than 80% of the covalently bound TCDD metabolites were removed, leaving only 0.016 p mole/mg of TCDD-equivalent radioactivity bound to DNA.

The amount of covalently bound TCDD equivalent to microsomal protein was 20.6 p moles/mg, indicating this binding occurred approximately 1,000 to 2,000 times more readily than the binding to DNA.

In the second study, Poland and Glover (1979) examined the *in vivo* covalent binding of TCDD (or metabolites) to rat liver macromolecules. In this study, tritium labeled ^{3}[H]TCDD, 95% chemically pure, was used (the impurity consisted of radiolabeled trichloro- and pentachlorodibenzo-p-dioxin). A dose of 7.5 mg/kg [1,6 ^{3}H]TCDD with specific activity of 39 Ci/mmole was administered intraperitoneally to Sprague-Dawley rats (approximately 90 uCi/rat). The dose level and duration of the experiment was selected on the basis of an acute toxicity study to obtain highest hepatic concentrations without substantial hepatic toxicity. The livers of the animals were pooled and

homogenized, and the homogenate was processed for fractionation of protein, ribosomal RNA, and DNA. These fractions were analyzed for radioactivity. The maximum unextractable radioactivity from liver protein fraction was 60 p moles of TCDD/mole of amino acid residue. The radioactivity associated with ribosomal RNA and DNA was very low. That associated with microsomal RNA corresponded to only 12 p moles of TCDD/mole of nucleotide residue, and that associated with DNA corresponded to only 6 p moles of TCDD/mole of nucleotide residue.

These two studies essentially demonstrated that TCDD was transformed to a reactive electrophile metabolite and showed significant covalent binding with cellular proteins, but less significant binding with DNA.

MUTAGENICITY OF 2,4,5-T

The mutagenicity of 2,4,5-T was evaluated by Ercegovich et al. (1977), employing the procedure of Ames using five strains of Samonella typhimurium without activation. The authors concluded that 2,4,5-T is non-mutagenic.

Anderson and Styles (1978) reported that 2,4,5-T at concentration ranges from 4 to 2500 ug per plate did not cause reversions in any of the four strains of Samonella typhimurium (TA 1535, TA 1538, TA 98, and TA 100) with or without microsomal activation. Several other investigators have reported negative responses with 2,4,5-T in bacterial test systems which have been summarized in a review by Grant (1979). Zetterberg (1978) found that 2,4,5-T increased the back mutation frequency in the histidine defective strain of Saccharomyces cerevisiae at pH values below 4.5, by approximately 300 fold at 40 mg/ml and 5000 fold at 60 mg/ml. However, the percent of survivors at the lower concentration was less than 5% and at the higher concentration less than 0.1%. The author concluded that 2,4,5-T is unlikely to cause mutations in a near neutral environment but oral administration may increase the risk of somatic mutation in the gastric tract where pH values are as low as 1.2. The 2,4,5-T used in these studies contained less than 1 ppm dioxins.

Majumdar and Golia (1974) fed Drosophila melanogaster males 1000 ppm 2,4,5-T for 15 days and found a small increase in the percentage of sex-linked recessive lethals by 0.61% over controls values of 0.05%. The herbicide was reported to contain no detectable amount of dioxin. Similar findings by Magnusson et al. (1977) also showed 2,4,5-T to be weakly mutagenic in Drosophila. In a parallel experiment, the known mutagen ethylmethanesulfonate at 250 ppm increased the incidence of sex-linked lethals by 13.65%. The CAG evaluated the negative

report by Vogel and Chandler (1974) concerning the mutagenicity of 2,4,5-T in the Drosophila melanogaster sex-linked recessive lethal tests as not providing sufficient data to classify the compound as postive or negative. The number of chromosomes analyzed by Vogel and Chandler was not large enough for a meaningful test. The negative report by Rasmuson and Svahlin (1978) is also inadequate to evaluate 2,4,5-T as a non-mutagen in Drosophila. These authors developed a test for detecting somatic cell mutagenesis and tested EMS, 2,4-D, and 2,4,5-T using it. EMS and 2,4-D were reported to be positive at concentrations of 500 and 25 ppm, respectively, but 2,4,5-T was reported to be negative. Since these results were obtained from the first set of experiments in a new test system, it is not possible to compare them in a meaningful way to other tests such as the sex-linked recessive lethal test in Drosophila melanogaster. Furthermore, the report suffered from other deficiencies such as a lack of information concerning compound purity and dose-response.

Fujita et al. (1975) reported chromosomal abnormalities in in vitro cytogenetics studies of human lymphocytes exposed to 10^{-7} to 10^{-4}M 2,4,5-T. Chromosome 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.

Yefimenko (1974) reported on an acute and chronic exposure to butyl ether 2,4,5-T in in vivo cytogenetics tests on gonadal and somatic tissue in male albino rats. Twenty-four hours after a single oral administration at doses of 1, 0.1, and 0.01 ug/kg, structural damage to bone marrow cell chromosomes was observed either as breaks or as true aberrations or rearrangements.

Chronic-exposure effects to the gonads were observed after exposure for 2-1/2 months to a dose of 0.1 ug/kg. The following effects were observed at the termination of the experiment (7 months): testicular atrophy, decreased sperm

count, desquamated tubules, and aberrant cells in the germinal epithelium. These effects persisted after exposure was terminated. Chromosomal aberrations were also observed during chronic dosing. The authors' methodology appears to be inadequate, however, and thus no valid conclusions can be drawn from this study. Majumdar and Hall (1973) reported that intraperitoneal injections of 2,4,5-T (containing no measurable amount of TCDD) into gerbils at concentrations of 350 mg/kg for 5 days produced 8.2, 4.6, and 1.8 percent incidences of chromatid gaps, chromatid breaks, and fragments, respectively, in bone marrow cells. Control values were given as 1.0% for gaps, 0.2% for breaks, and 0.2% for fragments. When the animals were treated at lower doses, no significant increases in chromosomal abnormalities were observed. Jensen and Renberg (1976) performed cytogenetic tests on mice injected with 2,4,5-T at 100 mg/kg. They reported no increase over control values in incidences of micronuclei in polychromatic or normochromatic erythrocytes, or polychromatic cells 24 hours or 0 days after the injection of the chemical. They were unable to confirm the cytogenic effect reported by Majumdar and Hall (1973), but pointed out that they used extremely high doses which might cause toxic effects leading to cell death and chromosomal fragmentation.

Renner (1979) reported that 2,4,5-T induces a weak positive response in the SCE test using Chinese hamster bone marrow cells. Four SCE's per cell were observed in the control animals compared to 7/cell at 100 mg/kg and 8/cell at 250 mg/kg. This report cannot be evaluated, however, because no information is provided concerning the route of administration, the number of animals used, the number of cells scored per animal, the purity and source of the compound, and whether or not the test was repeated.

Kilian et al. (1975) examined lymphocytes for chromosomal aberrations in industrial workers exposed to 2,4,5-T in a Midland Michigan plant and compared

them with a control group of workers prior to employment at the plant. They reported that there was no significant differences in the aberration rates among the control group (84 people) and those exposed for less than a year (16 people) or more than a year (17 people). However, the study did not indicate concentrations of 2,4,5-T workers might have been exposed to, and for each subject only about 20 cells were scored for chromosomal aberrations.

MUTAGENICITY OF TCDD

Hussain et al. (1972) reported positive results in three microbial test systems using a 99% pure TCDD sample obtained from the Food and Drug Administration (FDA). Reversion to streptomycin independence in Escherichia coli Sd-4 occurred with high frequency at a concentration of 2 ug TCDD/ml. Reversion at the histidine locus of Salmonella typhimurium TA 1532 occurred at concentrations between 2 to 3 ug/ml. This indicates that TCDD produces frameshift mutations by intercalation between base-pairs of DNA. A doubling in the frequency of prophage-induction was observed in E. coli K-39 exposed to TCDD. These studies were not performed with metabolic activation, indicating that TCDD is a direct-acting mutagen.

Seiler (1973) classified TCDD as a strong mutagen (where the ratio of number of revertants from treated plates per 10^8 bacteria divided by the number of spontaneous revertants per 10^8 bacteria is greater than 10) in the TA 1532 Salmonella strain which detects revertants through frameshift mutations. However, this report did not give the source or purity of TCDD, the concentration used in the assay, the toxicity of the compound where mutagenic activity occurs, or whether microsomal activation was necessary.

However, McCann (personal communication) tested TCDD to be negative in the standard plate test with strain TA 1532, with and without microsomal activation, and Nebert et al. (1976) also reported that TCDD was not mutagenic in the Salmonella in vitro assay. The differences between these laboratory results and those discussed above could be due to several factors such as treatment protocols, solubility problems of TCDD, and the high toxicity of this compound.

The Food and Drug Administration conducted a somatic in vivo cytogenetics screening study on TCDD in rats and got negative results (Green 1975). Separate experiments were performed with five multiple intraperitoneal doses or a single

oral dose regimen with sacrifice at 1 or 29 days. Toxicity, as indicated by slight body weight loss, was observed in the multiple dose study only at the highest dose used. This indicates that the dose levels may have been too low. Khera and Ruddick (1973) dosed male rats orally with 4 or 8 mg/kg/day TCDD for 7 days. These doses were acutely toxic and 20 survivors at the lower dose and 6 survivors at the high dose were mated after treatment seven times at 5-day intervals. Reproductive values indicated no occurrance of dominant lethal mutations. Green et al. (1977) studied the cytogenetic effects of TCDD on rat bone marrow cells. Male and female animals received 0.25, 1.00, 2.00, and 4.00 mg/kg TCDD by gavage twice a week for 13 weeks. The authors examined bone marrow cells at the end of treatment (approximately 50 cells per animal) for abnormalities. They concluded TCDD produces chromosomal aberrations in bone marrow cells but the effect is not one of great magnitude.

Chromosome analyses on 12 hospital patients exposed to TCDD in a July 1976 Seveso, Italy factory accident (Department of Health, Education, and Welfare, 1976) were examined for chromosomal lesions (gaps, chromatid and chromosome breaks, and rearrangements). These analyses presented at the DHEW-Subcommittee on Environmental Mutagenesis meeting, October 12, 1976 were of somatic cells from males and females ranging in age from 2 to 28. One patient had 19% cells (presumed blood cells) that were classified as having chromosomal lesions, another had 10%. The remaining patients had values comparable to control levels of 5 to 7%. Results from chromosome analyses of maternal peripheral blood, placenta, and fetal tissue in 17 women exposed to TCDD (amounts not given) who underwent spontaneous abortions were inconclusive. Reggiani (1977) reported that the frequency of spontaneous abortions in the Seveso zone did not significantly change nor did the incidence of malformations as a result of exposure to TCDD, even at the regions where the exposure was estimated through

soil analysis to be greater than 10 ug/kg. Similar conclusions were reached by Tuchmann-Duplesis (1977). Reports by both Reggiani (1977) and Tuchmann-Duplesis (1977) state no increase in abnormal cytological changes in tissues of aborted fetuses or in maternal blood in the Seveso zone during the exposure incidence to TCDD. However, these findings are poorly documented and complete experimental procedures and design used to evaluate the data were not available. Furthermore, it appears from these reports that only gross macroscopic alterations were sought and not microscopic lesions which are more difficult to assess. Such lesions are very dangerous in that they may survive and be carried to future generations.

CONCLUSIONS

There is some evidence that 2,4,5-T appears to be a weak mutagen causing point mutations. The best evidence for this is in Drosophila and Saccharomyces cerevisiae. However, evidence in Saccharomyces cerevisiae indicates the potency of the mutagenic effect may be related to the ionization of the carboxyl group of 2,4,5-T and is increased under more acidic conditions. At the present time, epidemiological evidence and cytogenetic studies for mutagenicity concerning TCDD are inconclusive. Also, the reported effects of TCDD as a "frameshift mutagen" are inconsistent. Because TCDD is structurally similar to acridines which produce frameshift mutations by intercalation in the DNA base-pairs, it is recommended that the ability of TCDD to induce forward mutations in systems such as mammalian cells in culture and the sex-linked recessive lethal tests in Drosophila be examined. Also, it is recommended that the mutagenic activity of TCDD be re-tested in bacteria using a series of both strains which detect frameshift and base-pair mutations.

IV. TOXICITY

ANIMAL TOXICITY

Toxicity of 2,4,5-T

Oral LD_{50} levels for 2,4,5-T as referenced by the National Institute of Occupational Safety and Health (1976), are shown in Table 2 below.

TABLE 2. ORAL LD_{50} LEVELS FOR 2,4,5-T

Species	LD_{50}
Dog	100 mg/kg
Rat	300 mg/kg
Guinea pig	381 mg/kg
Mouse	389 mg/kg

In a study cited by Rowe and Hymas (1954), no adverse effects were seen in dogs given 2,4,5-T by oral administration five times a week, for 90 days, at doses of 25 and 10 mg/kg. However, at a dose level of 20 mg/kg, all four dogs died and showed mild liver and kidney changes. A Dow Chemical Company internal report (1971) cited by EPA's 2,4,5-T Advisory Committee summarized a study using 2,4,5-T containing 0.5 ppm TCDD. The 2,4,5-T was fed to male and female rats for 90 days at dose levels of 0 to 100 mg/kg/day. No toxic effects were observed at doses of 30 mg/kg or lower. At a dose level of 100 mg/kg/day, some hematological effects and weight loss were noted, but toxic effects were described as minor and inconsistent.

Toxicity of TCDD

TCDD is one of the most toxic chemicals known to man. Oral LD_{50} values, shown in Table 3, range from 0.6 ug/kg orally for the male guinea pig to 275 ug/kg dermally for the rabbit. Deaths typically occur about a week or more after treatment.

Poland et al. (1971) cite a study in which rapid death in guinea pigs followed dermal application of the tarry residues from TCDD synthesis. When rabbit ears were painted with soil extracts contaminated with TCDD, hyperkeratosis and liver pathology were observed in the rabbits (Kimbrough 1974).

Kociba et al. (1978) conducted a 2-year chronic toxicity and oncogenicity study of TCDD in rats. In this study, the animals were maintained for 2 years on diets supplying 0.1, 0.01, and 0.001 ug TCDD/kg/day. Aside from carcinogenic effects, ingestion of 0.1 ug/kg/day caused increased mortality, decreased weight gain, slight depression of erythroid parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, along with increased serum activities of alkaline phosphatase.

In chronic and acute oral TCDD toxicity studies on several animal species, the liver, thymus, and spleen have consistently been the target organs. Liver damage, including necrotic and degenerative changes, lipid accumulation, and increased liver weight, have been observed in mice, rats, and guinea pigs following TCDD treatment (Vos et al. 1974, Jones and Greig 1975, Gupta et al. 1973, Goldstein et al. 1973, Kimmig and Schultz 1957). Liver damage was markedly greater in rats receiving a comparable dose (Gupta et al. 1973). It has been suggested that the fatty liver observed in mice may result from the starvation and loss of body weight that occur following TCDD treatment, or may be due to the induction of mixed-function oxidases (Jones and Greig 1975).

TABLE 3. LETHALITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN[a, b]

Species and sex	Route of administration	Time of death, days post administration	LD_{50} ug/kg	Dose ug/kg	Number deaths/ number treated
Rat, male	Oral	9-27	22	8	0/5
				16	0/5
				32	10/10
				63	5/5
Rat, female	Oral	13-43	45		
Guinea pig, male	Oral	5-34	0.6		
Guinea pig, male[c]	Oral	9-42	2.1		
Rabbit, mixed	Oral	6-39	115		
	Skin	12-22	275		
	Intraperitoneal	6-23 (all doses)	---	32	0/5
				63	2/5
				126	2/5
				252	2/5
				500	3/5
Dogs, male	Oral	9-15 (all doses)		300	0/2
				3000	2/2
Dogs, female	Oral	---		30	0/2
				100	0/2
Mice, male	Oral	---		114	---

[a]Responses to individual doses are given in those cases in which an LD_{50} could not be calculated.

[b]All values are from Schwetz et al. (1973), except those for male mice, which are from Vos et al. (1974).

[c]A sample that was more than 99% pure was used. All other tests except the mice study used TCDD that was 91% pure.

Atrophy of the thymus and spleen has also consistently been found in laboratory animals (Vos et al. 1974, Kociba et al. 1975, Gupta et al. 1973). Vos et al. (1973) reported that cell-mediated immunity was suppressed in guinea pigs and mice in TCDD-induced lymphoid depleted thymuses. Thigpen et al. (1975) found that mice receiving 1 ug/kg or more of TCDD by stomach tube once a week for 4 weeks had increased susceptibility to Salmonella infection. Female monkeys fed TCDD for 9 months showed hypocellularity of the bone marrow and lymph nodes as well as hypertrophy, hyperplasia, and metaplasia of the bronchial tree, epithelium, bile ducts, pancreatic ducts, and salivary gland ducts (Allen et al. 1977).

Other effects of TCDD ingestion include suppression of reproductive function in rats (Kociba et al. 1975) and disturbance of the hematopoietic system with occasional hemorrhaging in monkeys, rats, and mice (Allen et al. 1977, Kociba et al. 1975, Vos et al. 1974). TCDD interferes with the biosynthetic pathway of heme by inducing delta-aminolevulinic acid synthetase (-ALA), which results in hepatic porphyria in mice and rats (Goldstein et al. 1976). Increased urinary excretion of uroporphyrins has been observed in rat feeding studies (Kociba et al. 1977, Goldstein et al. 1976).

TOXICITY OF 2,4,5-T, 2,4,5-TRICHLOROPHENOL, AND TCDD IN HUMANS

The most consistently reported toxic effect of 2,4,5-T, 2,4,5-trichlorophenol, and TCDD to humans is chloracne, a disfiguring and long-term dermatitis. This has occurred in 2,4,5-T factory workers (Bauer et al. 1961, Poland et al. 1971), 2,4,5-trichlorophenol workers (Kimmig and Schulz 1957, Bauer et al. 1961, Bleiberg et al. 1964, Goldmann 1972), and laboratory workers accidentally exposed to TCDD (Oliver 1975). It has also been observed in exposed populations following the accidental production of TCDD in exothermic

reactions at chemical plants (Hay 1976, Kimmig and Schulz 1957, May 1973). In the 2,4,5-trichlorophenol plant accident in Seveso, Italy (1976), 300 to 500 grams of TCDD are believed to have been deposited in the most contaminated areas, with lesser amounts in surrounding areas (Hay 1976). In the other incidents, the level of TCDD present is not estimated.

There are scattered reports of hepatotoxic effects including abnormal liver function tests and pathological changes in the liver (Kimmig and Schulz 1957, Bauer et al. 1961, Bleiberg et al. 1964, Poland and Smith 1971). Eleven of 14 men exposed to TCDD during an exothermic reaction at a 2,4,5-trichlorophenol plant had abnormal liver function tests, but after 10 days without TCDD exposure, most tests were normal. Bleiberg et al. (1964) reported that 11 of 29 workers at a 2,4,5-trichloropenol plant had porphyria, but in a study of the same workers 6 years later, Poland et al. (1971) found no overt clinical cases of the disease. They suggested that the change may have been due to increased attention to worker safety or to a decrease in TCDD contamination.

Bauer et al. (1961) found one case of bloody urine in exposed workers. Hemorrhagic cystitis was reported in a 6-year-old girl who was playing in a horse area contaminated with 2,4,5-TCP (TCDD concentration of 31 to 33 ppm) (Carter et al. 1975). Other organ system effects that have been reported are gastrointestinal tract disturbances (Kimmig and Schulz 1957, Poland et al. 1971), neurological disturbances (Oliver 1975), and respiratory and cardiac disorders (Bauer et al. 1961). Psychological changes, including emotional instability, lethargy, diminished libido, and high manic scores on psychological tests, have also been noted (Poland et al. 1971, Oliver 1975, Bauer et al. 1961).

CARCINOGENICITY OF 2,4,5-T IN MICE

Muranyi-Kovacs et al. (Oral) Mouse Study (1976)

Inbred C3Hf and XVII/G strains of mice were used. They were given 100 mg/liter of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) in drinking water for 2 months, beginning at 6 weeks of age. (The 2,4,5-T product contained less than 0.05 ppm of 2,3,7 8-tetrachlorodibenzo-p-dioxin.) Thereafter, mice were given 2,4,5-T mixed with a sterile, commercial diet (UAR 1136) at concentrations of 80 ppm. It was not stated whether these levels represented maximum tolerated values. However, the authors indicated that this dose was 1/40 of the LD_{50}.

The mice were examined weekly for their general health and for the presence of tumors. They were allowed to die or were killed in extremis. Complete necropsies were performed and grossly altered organs were examined histologically. The urinary bladder was distended with fixative in mice suspected of having lesions.

C3Hf control male mice survived an average of 630 days; treated male mice, 511 days ($P = 0.001$); control females, 680 days; and treated females, 620 days. Survival times for XVII/G control male mice were 521 days; for treated male mice, 583 days; control females, 569 days; and for treated females, 641 days ($P = 0.01$).

Tumor presence in C3Hf female mice ingesting 2,4,5-T is indicated in Table 4. The results show that 12 of 25 C3Hf female mice (48%) ingesting 2,4,5-T developed tumors of all types, as compared to 9 of 44 control female mice (21%) ($P = 0.03$). No other strain-sex combination yielded statistically significant values, as evidenced by the data in Tables 4 and 5. Benign and malignant tumors were considered together in this study. The authors stated that the "hepatomas"

TABLE 4. TUMORS IN C3Hf MALE AND FEMALE MICE INGESTING 2,4,5-T[a]

Dose (ppm)	Sex	Lung	Liver	Leukemia	Other	Total	No. of mice with tumors
0	M	2	19	0	1[b]	22	21/43 (49%)
80 ppm	M	0	10	2	1[c]	13	12/22 (55%)
0	F	5	3	1	0	9	9/44 (21%)
80 ppm	F	0	4	3	6[d]	13	12/25 (48%)

[a]Effective number of mice surviving longer than 300 days or developing a tumor before 300 days of age.
[b]Pleomorphic salivary gland tumor.
[c]Fibrosarcoma; not included are one hyperplastic lesion of the urinary bladder and one hyperplastic lesion of the forestomach.
[d]Iosteogenic sarcoma, 2 sarcomas, 2 cutaneous tumors, and 1 tumor of the cervix.

TABLE 5. TUMORS IN XVII/G MALE AND FEMALE MICE INGESTING 2,4,5-T[a]

Dose (ppm)	Sex	Lung	Liver	Leukemia	Other	Total	No. of mice with Tumors
0	M	22	4	0	1[b]	27	25/32 (78%)
80	M	14	0	1	1[c]	16	15/20 (75%)
0	F	20	0	2	2[d]	24	21/40 (53%)
80	F	15	0	1	0	16	16/19 (84%)

[a]Effective number of mice are mice surviving longer than 300 days or developing a tumor before 300 days of age. In the XVII/G male mice, there was no significant difference between the number of tumor-bearing mice among treated animals as compared with controls, as shown in the table above.
[b]1 forestomach tumor.
[c]1 urinary bladder papilloma; not included are 2 hyperplastic lesions of the urinary bladders.
[d]2 hemangiomas.

and lung tumors, which were carcinomas and alveologenic adenomas, occurred in the same proportions in control and treated mice. Treated C3Hf females had several tumors at sites not found in the controls. The authors reported a significant increase in total tumors in one strain and one sex of rats at one dose level. In reaching this conclusion, they used the Peto method and distinguished between incidental and nonincidental tumors.*

To clarify questions concerning the design, execution, and interpretation of this study, the CAG communicated with the principal author at the Curie Foundation, Marseilles, France. From this discussion and from the published account of this discussion it is concluded that: 1) this study was very insensitive because insufficient numbers of animals were used in the treatment groups; 2) the care of the animals was inadequate; 3) because the dose used, 80 ppm, was only 1/40 of the LD_{50}, and appears to be less than the maximum tolerated dose; 4) histologic examination of all animal tissues was not performed; and 5) only macroscopically altered tissues were examined histologically. In addition, the author recommended that more adequate studies be conducted in a greater number of species. Because of the severe deficiencies in the study, the CAG concluded that this study does not provide significant evidence for either the carcinogenicity or non-carcinogenicity of 2,4,5-T.

Muranyi-Kovacs et al. (Subcutaneous) Mouse Study (1977)

In this study, the authors administered 2,4,5-T to two strains of mice, C3Hf and XVII/G. Subcutaneous injections were given at 10 mg/kg of body weight in an

*These results are not considered to be evidence of an oncogenic response because there is no valid basis for grouping tumors at all sites or for distinguishing between incidental and nonincidental tumors. The author did not report any increases in tumors for any specific target site.

aqueous solution on days 1, 3, 6, and 10 of the animals' lives. The sex, strain, number of animals, survival time, and animals with tumors are shown in Table 6.

TABLE 6. SURVIVAL TIME AND TUMOR INCIDENCE IN 2,4,5-T TREATED MICE[a]

Strain	Sex	Dose mg/kg	Number of animals	Survival time tumor-bearing	Percent of animals with tumors
XVII/G	M	0	32	25/32	78
	M	4 x 10	15	13/15	87
XVII/G	F	0	40	21/40	53
	F	4 x 10	15	4/15	25
C_3H/f	M	0	43	21/43	49
	M	4 x 10	11	4/11	36
C_3H/f	F	0	44	9/44	21
	F	4 x 10	14	3/14	21

[a]TCDD content of 2,4,5-T was less than 0.05 ppm.

As indicated, there is no observed increased incidence of tumor-bearing animals as compared to the treated animals of both sexes and strains. However, this study was so incompletely reported that the details of the methodology cannot be discerned. In addition, this study was very insensitive because insufficient numbers of animals were used in the treatment groups, and only a few subcutaneous doses were administered. Therefore, these studies do not provide significant evidence for either the carcinogenicity or non-carcinogenicity of 2,4,5-T.

Innes et al. (Bionetics Laboratories 1968) (Oral) Mouse Study (1969)

The maximum tolerated dose of 2,4,5-T* was given to two hybrid strains of mice, (C57BL/6 x C3H/Anf)F_1, B6C3F1 designated as "strain X," and (C57B/6 x AKR)F_1, B6AKF1 designated as "strain Y." There were 18 treated mice and 18 untreated control mice of each strain and each sex. Each day, beginning at 7 days of age, 21.5 mg/kg of 2,4,5-T in 0.5% gelatin was administered by stomach tube. After weaning at 28 days of age, 60 ppm of 2,4,5-T was mixed directly in the diet and provided ad libitum. Treatment was continued for approximately 18 months.

At this time mice were killed and grossly examined both internally and externally in the areas of the neck glands and the thoracic and abdominal cavities. Histologic examination of major organs and all grossly visible lesions was performed. Thyroid glands were not examined. The postmortem results are given in Tables 7 and 8.

The results of the oral mouse study indicate that there was no significant difference between the 2,4,5-T-treated and control groups of mice with respect to tumors at specific sites, or total number of tumor bearing animals. This study, however, does not provide significant evidence for the non-carcinogenicity of 2,4,5-T because of certain defects in its design. The use of small numbers of animals and the duration of the study, which was only 18 months rather than the entire lifetime, made the study relatively insensitive for detecting an oncogenic effect.

* The Bionetics study did not report the level of TCDD contamination in the 2,4,5-T used. The 2,4,5-T used in a reproductive study conducted at approximately the same time as the Bionetics study was reported to contain 30 ppm TCDD. It is possible that the contaminant of 2,4,5-T used in the Bionetics study was the same as that of the 2,4,5-T used in the reproductive study. However, this conclusion is far from certain without actual chemical analysis of the 2,4,5-T used in the Bionetics study.

TABLE 7. MALE AND FEMALE MICE INGESTING 2,4,5-T WITH TUMORS OF VARIOUS ORGANS

Strain	Dose (ppm)	TUMOR TYPE Reticulum cell sarcoma		Pulmonary adenoma & carcinoma		Hepatoma	
		Male	Female	Male	Female	Male	Female
"X" matched	0	0/15	1/18	2/15	1/18	3/15	0/18
"X" pooled	0	5/79	4/87	5/79	3/87	8/79	0/87
"X"	60	1/18	0/18	1/18	1/18	4/18	0/18
"Y" matched	0	0/18	1/15	3/18	0/15	0/18	0/15
"Y" pooled	0	1/90	3/82	10/90	3/82	5/90	1/82
"Y"	60	2/18	1/18	0/18	0/18	1/18	0/18

TABLE 8. MALE AND FEMALE MICE INGESTING 2,4,5-T WITH TUMORS AT ALL SITES

		Male	Female
"X" matched	0	5/15 (33%)	2/18 (11%)
"X" pooled	0	22/79 (28%)	8/87 (9%)
"X"	60	6/18 (33%)	1/18 (6%)
"Y" matched	0	3/18 (17%)	1/15 (7%)
"Y" pooled	0	16/90 (18%)	7/82 (9%)
"Y"	60	3/18 (17%)	2/18 (11%)

Innes et al. (Bionetics Laboratories 1968) (Subcutaneous) Mouse Study (1969)

2,4,5-T in dimethysulfoxide (DMSO) was given as a single subcutaneous injection (215 mg/kg) to two strains of male and female mice (same strains as in the oral study) at approximately 28 days of age. The mice were observed for approximately 18 months. At that time mice were killed and examined grossly, both internally and externally, in the areas of the neck, glands, and thoracic and abdominal cavities. Histologic examinations of all major organs, as well as all grossly visible lesions, were made. Thyroid glands were not examined. The authors stated that histopathologic data did not show a statistically significant difference between the 2,4,5-T-treated and control groups either with respect to tumors at specific sites, or total number of tumor-bearing animals. However, this study suffered from the same deficiencies as the Innes et al. oral study. In addition, single subcutaneous dose studies are considered to be highly insensitive for detecting an oncogenic response. Therefore, the CAG does not consider this study to provide significant evidence of the non-oncogenicity of 2,4,5-T.

CARCINOGENICITY OF 2,4,5-T IN RATS

Kociba et al. (Oral) Rat Study (1978, 1979)

The cancer bioassay study of 2,4,5-T in rats (TCDD content was not detectable in 2,4,5-T with a detection limit of 0.33 ppb) was performed by Kociba et al. (1978, 1979). In this study, one group of 86, and three groups of 50 Sprague-Dawley (Spartan substrain) rats of each sex were administered 0, 3, 10, and 30 mg/kg body weight/day, respectively, via the diet, for periods up to 2 years. There is some question as to the actual dosage. Adequate information is lacking to show that the amount of 2,4,5-T found in the food by chemical analysis actually remained constant in a given feed lot during the entire period of consumption of that given lot. Based on the analytical data, the actual doses given were slightly lower than the nominal doses.

There are certain aspects of this study which reduced its sensitivity for detecting a carcinogenic response. First, there was a very high early mortality among all groups of males and females (Tables 9 and 10). The mortality data show that at the termination of the study more than 50% of the females had died in the control group as well as in each of the treated groups (the mortality was 76% in the 10 mg/kg female group). Among males, mortality was approximately 92% in the controls and ranged from 78% to 92% in the treated groups. Very high mortality in both males and females was observed as early as 21 months. This early mortality is important because it reduces the number of animals at risk for late developing tumors.

The second factor which reduced the sensitivity of this study was the relatively high incidence of spontaneous tumors in some organ sites in the controls. For example, among 86 control males, three hepatocellular carcinomas and four hepatocellular neoplastic nodules were found.

The data reported by Kociba et al. are presented in Table 11:

TABLE 9. CUMULATIVE MORTALITY DATA OF MALE RATS MAINTAINED ON DIETS CONTAINING 2,4,5-T FOR 2 YEARS

	Dose Level (mg/kg/day)			
	0	30	10	3
	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)
Original no. in group	86	50	50	50
Days on test				
0-30	0	0	0	0
31-60	0	0	0	0
61-90	1(1.2)	0	0	0
91-120	1(1.2)	0	0	0
121-150	1(1.2)	0	0	0
151-180	1(1.2)	0	0	0
181-210	1(1.2)	0	0	0
211-240	1(1.2)	0	0	0
241-270	1(1.2)	0	1(2.0)	0
271-300	2(2.3)	0	1(2.0)	0
301-330	2(2.3)	0	1(2.0)	0
331-360	2(2.3)	0	1(2.0)	1(2.0)
361-390	2(2.3)	2(4.0)	2(4.0)	2(4.0)
391-420	5(5.8)	2(4.0)	2(4.0)	3(6.0)
421-450	6(7.0)	2(4.0)	4(8.0)	4(8.0)
451-480	9(10.5)	4(8.0)	9(18.0)	6(12.0)
481-510	10(11.6)	6(12.0)	12(24.0)[a]	10(20.0)
511-510	16(18.6)	8(16.0)	22(44.0)[a]	12(24.0)
541-570	23(26.7)	11(22.6)	24(48.0)[a]	14(28.0)
571-600	32(37.2)	16(32.0)	29(58.0)[a]	23(46.0)
601-630	47(54.6)	19(38.0)	37(74.0)[a]	30(60.0)
631-660	67(77.9)	24(48.0)[a]	38(76.0)	32(64.0)[a]
661-690	74(86.0)	27(54.0)[a]	42(84.0)	34(68.0)[a]
691-720	77(89.5)	32(64.0)[a]	45(90.0)	38(76.0)[a]
721-728	79(91.7)	39(78.0)[a]	46(92.0)	40(80.0)[a]
Total no. of rats studied	86	50	50	50

[a]Statistically significant difference from control values by Fisher's Exact Probability Test, $P < 0.05$.

TABLE 10. CUMULATIVE MORTALITY DATA OF FEMALE RATS MAINTAINED ON DIETS CONTAINING 2,4,5-T FOR 2 YEARS

	Dose Level (mg/kg/day)			
	0	30	10	3
	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)
Original no. in group	86	50	50	50
Days on test				
0-30	0	0	0	0
31-60	0	0	0	0
61-90	0	0	0	0
91-120	0	0	0	1(2.0)
121-150	0	0	0	1(2.0)
151-180	0	0	1(2.0)	1(2.0)
181-210	1(1.2)	0	1(2.0)	1(2.0)
211-240	1(1.2)	0	1(2.0)	1(2.0)
241-270	1(1.2)	0	1(2.0)	1(2.0)
271-300	1(1.2)	1(2.0)	1(2.0)	1(2.0)
301-330	2(2.3)	1(2.0)	3(6.0)	2(4.0)
331-360	2(2.3)	2(4.0)	3(6.0)	2(4.0)
361-390	2(2.3)	3(6.0)	3(6.0)	3(6.0)
391-420	4(4.6)	3(6.0)	4(8.0)	3(6.0)
421-450	5(5.8)	3(6.0)	5(10.0)	3(6.0)
451-480	9(10.5)	7(14.0)	8(16.0)	5(10.0)
481-510	12(14.0)	9(18.0)	13(26.0)[a]	7(14.0)
511-540	18(21.0)	11(22.6)	14(28.0)	10(20.0)
541-570	20(23.2)	12(24.0)	15(30.0)	12(24.0)
571-600	25(29.1)	15(30.0)	16(32.0)	16(32.0)
601-630	29(33.7)	18(36.0)	21(42.0)	20(40.0)
631-660	34(39.5)	24(48.0)	25(50.0)	23(46.0)
661-690	41(47.7)	26(52.0)	33(66.0)[a]	26(52.0)
691-720	46(53.5)	27(54.0)	35(70.0)[a]	29(58.0)
721-732	46(53.5)	28(56.0)	38(76.0)[a]	29(58.0)
Total no. of rats studied	86	50	50	50

[a]Statistically significant difference from control values by Fisher's Exact Probability Test, $P < 0.05$.

TABLE 11. STRATIFIED SQUAMOUS CELL CARCINOMA OF THE TONGUE OF SPRAGUE-DAWLEY RATS FED WITH PURIFIED 2,4,5-T

	Kociba 2,4,5-T Controls	2,4,5-T dosage in mg/kg/day			
		30 (P-value)[a]	10	3	Test for Trend[b]
Males	1/83	4/49 (P = 0.063)	0/46	1/50	< 0.03
Females	0/83	1/49 (P = 0.371)	0/48	0/48	N.S.[c]

[a]P values determined by Fisher's Exact Test (one-tailed).

[b]Cochran's test for trend, one-tailed, scoring = 0, 1, 2, 3.

[c]N.S. = not significant at P = 0.05.

The increase in squamous cell carcinoma of the tongue in males at the 30 mg/kg/day dose level is marginally statistically significant (P = 0.063). Also, the dose-related trend for the incidence of tongue tumors in males is statistically significant in the Cochran-Armitage Test (P < 0.03).

Examination of male Sprague-Dawley rats in the Dow studies (Spartan substrain) for historical controls found the following incidence of squamous cell carcinomas of the tongue as illustrated in Table 12 (taken from selected Tables provided to EPA by Dow which summarize the results of six Dow studies).

TABLE 12. TONGUE TUMOR INCIDENCE IN HISTORICAL CONTROLS

	Tongue carcinomas/ no. examined	%
1. Kociba TCDD study	0/76	0
2. Kociba 2,4,5-T study	1/83	1.2
3. Dow Historical Control Study No. 4	1/63	1.6
4. Dow Historical Control Study No. 5	1/75	1.3
5. Dow Historical Control Study No. 6	1/49	2.0
Total	4/346	1.2

A comparison of the 4/49 tongue carcinomas vs. 4/346 historical controls yields a value of ($Z = 3.26$) $P < 0.001$, one-tailed test.

Dr. Robert Squire, pathologist at the Johns Hopkins School of Medicine and consultant to the Carcinogen Assessment Group, performed an evaluation of histopathological slides from Dow Chemical Company's 2-year rat feeding studies of 2,4,5-T (see Appendix B). A comparison of his findings to Dr. Kociba's histopathological evaluation of tongue lesions are summarized in Table 13. The histological analysis of Dr. Robert Squire revealed the occurrence of squamous cell carcinomas of the tongues in 5 of 48 high dose males as compared to the 4 of 49 high dose males reported by Dr. Kociba. Dr. Squire and Dr. Kociba both found one tongue carcinoma out of 83 controls. The histopathologic evaluation of the extra tongue carcinoma observed by Dr. Squire in the high dose group was confirmed by Dr. Goodman, pathologist, who reviewed six slides with tongue lesions in male rats from the Dow Chemical study. Five of these were squamous

cell carcinomas of the tongue in high dose males and 1 was in a control male (Goodman 1980).

The increase in squamous cell carcinomas of the tongue in males at the 30 mg/kg/day dose level is statistically significant (P = 0.025) compared to matched controls when using Drs. Squire's and Goodman's diagnoses. These results provide highly suggestive evidence of the carcinogenicity of essentially pure 2,4,5-T.

TABLE 13. DRS. SQUIRE'S AND KOCIBA'S REVIEW OF DOW 2,4,5-T ORAL RAT STUDY (8/15/80)
Sprague-Dawley Rats - Spartan Substrain (2 yrs.)

MALES

Tissue and Diagnosis	Dose Levels (mg/kg/day)							
	0 (control)		3		10		30	
	S	K	S	K	S	K	S	K
Tongue								
Squamous cell carcinoma	1/83	1/83	1/50*	1/50	0/46*	0/46	5/48 (P = 0.025)	4/49 (P = 0.063)

*Dr. Squire examined all slides from the middle and low dose described by Dow (original report) as exhibiting any lesions, but did not review tongue slides that Dow described as having no lesions. The incidence numbers for low and medium dose levels in this tble represent lthis combined review incidence (i.e., Dow's tongue diagnoses confirmed by Dr. Squire).

S = Dr. Squire's histopathologic evaluation

K = Dr. Kociba's histopathologic evaluation

The question arises whether these squamous cell carcinomas of the tongue could have been induced by any TCDD contamination which was present below the level of detection. Assuming TCDD was present at the level of detection (0.33 ppb), the amount of TCDD daily intake in the 2,4,5-T was estimated at less than 10 pg/kg/day. A second long-term TCDD study by Kociba (1978) on TCDD in Sprague-Dawley rats, also showed increased squamous cell carcinoma of the tongue in males. The results from the TCDD study are shown in Table 14.

TABLE 14. KOCIBA (1978) STUDY ON TCDD IN MALE SPRAGUE-DAWLEY RATS

		pg/kg/day TCDD		
Site	Control	100,000	10,000	1,000
Tongue-stratified squamous cell carcinoma	0/76[a]	3/50	1/50	1/50
Fisher's Exact Test (one-tailed)		P = 0.06	N.S.[b]	N.S.[b]
Test for trend exact test			P = 0.01	

[a]Only 76 of 85 tongues were examined microscopically.

[b]N.S. = not significant at P = 0.05.

Two exact probability tests both show statistical significance at the P = 0.06 level. The high dose response of 3/49 tumors at 100 ng/kg/day is significant at the P = 0.06 level, and the exact test for trend has a P-value = 0.01. Thus, the Kociba TCDD study provides suggestive evidence of a carcinogenic effect in the tongues of males.

A comparison of the two Kociba studies at comparable TCDD dose levels for comparable effects can only be made approximately. At 30/mg/kg/day 2,4,5-T, the

TCDD would have been present, if present at all, at a level below 10 pg/kg/day. This group showed in response of 4/49 (using Kociba's diagnoses). For the TCDD study, the comparison of 1/50 at 1,000 pg/kg/day TCDD vs. 5/49 (at 10 pg/kg/day), was not statistially significant (P = 0.36, two-tailed test). This leads us to conclude that the carcinogenic effect on the tongue exhibited in the 2,4,5-T study was at least partly and probably totally due to the 2,4,5-T and not to the TCDD contaminant. But in another way, the TCDD dose required for 3/50 tongue tumors was 100,000 pg/kg/day.

Based on the dose response slope of tongue tumors from the TCDD study, the estimated number of tongue tumors from the 10 pg/kg/day TCDD contaminant in the 2,4,5-T study would have been 1×10^{-5}. Even considering the historical control group experience, the expected number would be less than 0.6.

Thus, based on the above analysis, if the TCDD contamination level of 2,4,5-T in the Kociba study was not greater than 0.33 ppb, then this study of 2,4,5-T provides highly suggestive evidence of the carcinogenicity of 2,4,5-T in rats.

Leuschner et al. (Oral) Rat Study (1979)

Leuschner et al. (1979) investigated the chronic effects of commercial grade 2,4,5-T (containing 0.05 ppm TCDD) on Sprague-Dawley (SIV50) rats. The study used four groups of 60 male and 60 female rats from the F_1 generation of a three-generation reproduction study in which the dams received 2,4,5-T at 0, 3, 10, and 30 mg/kg body weight/day in the diet. From 6 weeks (42 days) of age, rats in the four groups were placed on the same feeding regimen as their mothers. The treatment continued for the duration (130 weeks) of the experiment. Three groups received 2,4,5-T dissolved in acetone, which was poured over a small quantity of feed and then mixed after the evaporation of the acetone. One group, identified in the report as the pre-mix controls, was given

only acetone in the diet. A fresh diet was prepared every 7 days.

Additional groups of 60 male and 60 female Sprague-Dawley rats served as untreated controls. Rats in this group were supplied at 6 weeks of age by the same source that had supplied the F_0 generation of the three-generation study. During the experiment, clinical signs, body weights, and consumption of food and water, were monitored at regular intervals. Urinalyses were performed and hematological and clinical chemistry parameters were determined for 10 rats from each group at regular intervals. The same rats were used for measurements throughout the experiments; the authors found no effects attributable to 2,4,5-T in any of these observations. At 13 weeks, 10 rats were sacrificed from each group and examined leaving 50 animals of each sex for long-term exposure. Rats that died, were moribund, or killed during the experiment, and all surviving rats killed after 130 weeks, were necropsied. All major tissues of all animals, except for tissues of the survivors dosed at 3 mg/kg/day, were examined histopathologically.

The authors reported that they found no evidence that the test compound had a toxic or carcinogenic effect on either male or female rats. The type and incidence of lesions observed were considered normal in old-age breeding rats of the test strain. However, a statistically significant increase in interstitial cell tumors of the testes in the high dose group of males ($P = 0.014$), as well as a significant dose-related trend ($P < 0.01$) for these tumors was observed when comparison is made to the incidence of these tumors in the pre-mix control animals (Table 15). The significance of these results disappeared when comparison was made to the untreated control group, which had an incidence of testicular tumors higher than that in the high dose group. The incidence of testicular tumors in the untreated controls (22/50 or 44%) is very significantly higher ($P < 0.01$, using a one-tailed Fisher Exact Test) than that in the pre-mix

controls (12%). Because of this difference, the two control groups cannot be pooled for comparision with the treated groups. Clearly, a compound-related increase can be shown only if the pre-mix control group alone is used as a point of comparison with the treated groups.

Because of the experimental design, the pre-mix controls are the appropriate comparision group. However, in this situation there is a question of whether the pre-mix or untreated controls manifest an atypical spontaneous rate for testicular tumors. The results are not statistically significant when the incidence of testicular tumors in the high dose group is compared to the incidence of testicular tumors in historical controls provided by Leuschner (1979). In each of the four sets of historical controls [designated tests A_1 - 20/50 (40%), A_2 - 24/90 (26.6%), A_3 - 17/50 (34%), A_4 - 32/100 (32%) the incidence of testicular tumors is comparable to that in the high dose group 16/50 (32%). However, these historical controls were untreated controls rather than vehicle (acetone) treated controls, and therefore may not be appropriate for comparision. Moreover, in the low dosage group, while 14 testicular masses were observed macroscopically, only six of those masses were examined microscopically. All six masses examined microscopically were testicular tumors. If the remaining eight masses were examined microscopically and were also proven to be testicular tumors, the dose-related trend would no longer be significant.

TABLE 15. INTERSTITIAL-CELL TUMORS OF TESTES IN MALE RATS

Dose	Rats with tumors	P-Value[a]	Percent animals with tumors
untreated controls	22/50		44%
pre-mix controls	6/50		12%
10/mg/kg/day group	12/50	N.S.[b]	24%
30 mg/kg/day group	16/50	0.014	32%

[a]P - Value calculated with Fisher Exact Test (one-tailed).
[b]N.S. = not significant at P = 0.05.

This study suffers from the following limitations: 1) the maximum tolerated dose was apparently not used; 2) the observed testicular tumors are often associated with old-age with variable incidences; 3) testicular masses were reported in 14/28 of the animals exposed at the low dose (3 mg/kg/day), but only six of these masses were diagnosed microscopically; and 4) the difference in the incidences of testicular tumors in the two contol groups makes interpretation of the significance of the testicular tumor incidence in treated groups uncertain.

In conclusion, the significance of the results concerning the incidence of testicular tumors is uncertain. In addition, this test cannot be considered a valid negative study of 2,4,5-T because the highest dose used was less than the maximum tolerated dose. This reduced the sensitivity of the test for detecting the possible oncogenic effects of 2,4,5-T.

The tongue, which was a site of increase in tumor incidence in the Kociba studies was not initially examined microscopically in the Leuschner study. Therefore, the CAG requested the histopathological examination of tongue lesions

(cutting the tissues horizontally). Due to some misunderstanding, it appears that longitudinal sectionals were made (see letter from Wade Richardson to Dr. Charalingayya Hiremath, Appendix E).

The tongues of the male rats treated with 30 mg 2,4,5-T/kg body weight/day in the food and those of the untreated male rats (without premix) were investigated histopathologically after haematoxylin-eosin staining. Longitudinal sections reaching from the retrolingual region to the tip of the tongue were prepared. Each of 8 gradual sections of the tongue were investigated. the mucosal epithelial thickness of the treated and untreated rats was compared.

The histopathologic examination of the tongue did not reveal any neoplastic lesions that were observed by Kociba in his 2,4,5-T study at the same dose level feeding studies (Appendix D). Although the adequacy of the histopathological examination of the tongues in the Leuschner study is not clear at the present time, the unusual site of tumor formation in the tongue in the Kociba study and the apparent lack of reproducability of this tongue tumor response in the Leuschner study reduced our judgment of the strength of evidence of the carcinogenicity of pure 2,4,5-T provided by the Kociba study from substantial to highly suggestive.

CARCINOGENICITY OF (2,4,5-TRICHLOROPHENOXY)PROPIONIC ACID (SILVEX)

Innes et al. (Bionetics Laboratories 1968) (Oral) Mouse Study (1969)

Innes et al. (Bionetics Laboratories 1968) (1969), under the sponsorship of the National Cancer Institute, investigated the carcinogenicity of silvex in two studies with mice, one oral and the other subcutaneous. In the oral study, groups of 18 B6C3F1 and 18 B6AKF1 mice of each sex were given the test substance daily in 0.5% gelatin by oral gavage at 46.4 mg/kg body weight beginning at 7

days of age and continuing until they reached 28 days of age. At that time, 121 ppm of silvex was administered daily in the diet. This study was carried out for approximately 18 months. Mice were housed by sex, up to six in a cage, and were given food and water ad libitum. All animals were observed daily for clinical signs and weighed weekly. The doses administered were the maximum tolerated doses, which had been selected from pre-chronic toxicity studies performed before the initiation of the chronic study. The moribund mice were killed, necropsied, and selectively examined microscopically, while surviving animals were killed at approximately 18 months and necropsied. Heart, lungs, liver, spleen, kidneys, adrenals, stomach, intestines, genital organs, and tissue masses were placed in formalin. They were later sectioned, stained with hematoxylin and eosin, and examined microscopically. All but five mice, three B6C3F1 male and two B6AKF1 male or female, survived 18 months. Table 16 identifies the types of tumors and the groups in which they were found.

TABLE 16. TUMORS IN MICE EXPOSED ORALLY TO SILVEX

Type of Tumor	B6C3F1 Mice		B6AKF1 Mice	
	M	F	M	F
Reticulum-cell sarcoma, type A	1	1	0	0
Pulmonary adenoma	1	0	1	0
Hepatoma	5	0	0	0
Mammary adenocarcinoma	0	1	0	0
Angioma	1	0	0	0
Gastric papilloma	0	2	0	0
Adrenal cortical adenoma	0	0	0	1

There was no significant increase in the incidence of neoplasms in B6C3F1 or B6AKF1 male or female mice administered silvex orally. However, by current National Cancer Institute guidelines, there are a number of deficiencies in these studies: 1) only a single dose level was administered, 2) the number of animals in the treatment group (18) was too small, and 3) the experiment was terminated after only about 18 months. Because of these deficiencies, the test was relatively insensitive for detecting a possible oncogenic effect of silvex and therefore cannot be considered as significant evidence of the non-carcinogenicity of silvex.

Innes et al. (Bionetics Laboratories 1968) (Subcutaneous) Mouse Study (1969)

In this study, groups of 18 B6C3F1 and 18 B6AKF1 mice of each sex, approximately 28 days of age, were given a single subcutaneous injection of 215 mg/kg body weight of 2-(2,4,5-trichlorophenoxy)propionic acid (silvex, supplied by Methson Coleman Bell Co.) suspended in dimethyl sulfoxide (DMSO), and observed for 18 months. Procedures similar to those described above for the Bionetics oral study were followed for animal housing, care, observation, necropsies, selection of tissues, and preparation of histologic slides. All animals, except two B6C3F1 male mice, survived 18 months. Seven tumors were diagnosed in the 18 male B6C3F1 mice examined. The tumors were: two reticulum-cell sarcomas, type A; two pulmonary adenomas; one hepatoma; and two hemangiomas. Only one tumor, a gastric papilloma, was diagnosed in one of the 18 female B6C3F1 mice examined. A hepatoma was found in one of the male B6AKF1 mice examined. These incidences were comparable to those seen in the control animals. There was no significant increase in the incidence of neoplasms in B6C3F1 or B6AKF1 male or female mice by subcutaneous injection. However, there

were a number of deficiencies in this study: 1) only one subcutaneous injection was given, 2) the number of animals in the treatment group (18) was too small, and 3) the experiment was terminated after only 18 months. Because of these deficiencies, the test was relatively insensitive for detecting an oncogenic effect of silvex.

Dow Chemical Company (Oral) Rat Study, summarized in Mullison (1966) and Gehring and Betso (1978)

Groups of Wister rats (30 males and 30 females in each group) were fed diets containing 0.0, 0.03, 0.003, and 0.001% Kurosol®SL (potassium salt of silvex) for up to 24 months. Administration of the test compound began at 50 days of age. Animals were sacrificed at 12 and 18 months so that the group sizes at the end of the 2-year study could not have been more than 21 or 22 per sex; they may have been even smaller. However, the size of the groups at the end of the study cannot be exactly determined since no data were provided on the extent to which animals, other than the ones sacrificed, died before the end of the study.

There was no evidence of a toxic effect or reduced survival in female rats administered any dose compared to controls. Therefore, it does not appear that the females were administered the maximum tolerated dose. Since high dose males exhibited a significant decrease in average body weights, it appears that they were administered a maximum tolerated dose.

No significant increase in tumors was reported. However, because small groups of animals were used and the maximum tolerated dose was apparently not used in the high dose females, this study cannot be considered as significant evidence of the non-carcinogenicity of silvex in rats.

Dow Chemical Company (Oral) Dog Study (1965), summarized in Mullison (1966) and Gehring and Betso (1978)

Groups of beagle dogs (four males and four females in each group) were fed diets containing 0.0, 0.056, 0.019, and 0.0056%. Kurosal®SL (potassium salt of silvex). One male and one female of each group were killed at 12 months. The remaining animals were killed at the end of the 2-year period.

No incidence of tumors was observed. However, this study does not constitute a valid cancer study because its duration was far less than the life expectancy of a beagle dog and the sizes of the animal groups were exceedingly small. Therefore, this study provides no evidence of the lack of carcinogenicity of silvex in dogs.

Kociba et al. (Oral) Rat Study (1977, 1978)

Although this study was reported in published form in Toxicology and Applied Pharmacology (1978), a fuller version was submitted in an unpublished report (Kociba et al., Dow Chemical Company, September 28, 1977).

In this study, groups of 50 Sprague-Dawley rats (Spartan substrain) of each sex were maintained for up to 2 years on diets providing 0.1, 0.01, or 0.001 ug/kg/day TCDD. Vehicle control groups comprised 86 animals of each sex. The test was appropriately conducted with the high dose group at a level which induced signs of tissue toxicity, reduced weight increments in both sexes, and shortened lifespans in female rats. Clinical tests performed at intervals during the study monitored organ specific toxicity, particularly of the liver. Pathologic examinations included histopathologic evaluation of all major tissues in both the high dose and control animals, but only of selected tissues identified as possible target organs and suspect tumors in lower dose groups. This approach is suitable for the identification of a carcinogenic effect, but does not determine actual tumor incidences in all groups except in those organs identified as target organs. It, therefore, is adequate to define dose-response relationships only in these target organs. Tissues examined from most animals in all dose groups included liver, lungs, kidneys, urinary bladdar, tongue, brain, testes/ovaries, and prostate/uterus. For these tissues, a quantitative analysis can be performed using the actual number of tissues examined histopathologically for animals at risk. For other tissues (excluding skin, mammary glands, and nasal turbinates/hard palate), actual tumor incidence cannot be evaluated for the two lower doses. For skin and mammary glands, the number of animals necropsied is the appropriate denominator to determine incidence, because detection of these tumors is based on observation of the tumor at

necropsy.

A laboratory audit of this study by Spencer and Woodrow, Hazard Evaluation Division, Office of Pesticide Programs, U.S. Environmental Protection Agency did not reveal significant new information. Reviewers concluded that the study was properly conducted, adhering to the accepted procedures (memorandum from Spencer and Woodrow to Diana Reisa, and Warnick Project Manager for 2,4,5-T).

Based on data reported for food consumption, body weight, and dietary level of TCDD, the daily doses were reasonably constant for most of the study, although somewhat below the value expected in most groups during the third month.

High early mortality was observed in all groups in this study but was only statistically significant in the high dose group. The survival curves show progressive mortality beginning as early as the 12th month and leading to 50% mortality by 21 months.* The effects of this early mortality are a reduction in expected tumor incidence because of a truncated latency period, and a reduction in sensitivity of the study because of a reduction in number of animals at risk during the time of expected tumor manifestation. Cumulative mortality and interval mortality rates are given in Tables III-7 to 10 of Appendix A (Clement Associates 1979).

The results of this study provide substantial evidence that TCDD is carcinogenic in rats. TCDD induced a highly statistically significant increase of both hepatocellular carcinomas and hepatocellular neoplastic nodules in

*In the 0.001 group of males, 44% mortality was as early as 18 months. The mortality patterns were analyzed by the Whitney-Wilcoxon test and Kolmogorov-Simonov test. These tests show that mortality was significantly higher in the high dose females than in controls, and while indications of increased mortality were found in other groups, they were not part of a consistent pattern.

female rats at doses of 0.1 nd 0.01 ug/kg/day (2200 and 2 0 ppt in the diet, respectively). The increase of hepatocellular carcinomas alone, in the high dose females, was also highly significant. In addition, at the highest dose level, TCDD induced a statistically significant increase in stratified squamous cell carcinomas of the hard palate and/or nasal turbinates in both males and females, squamous cell carcinomas of the tongue in males, and keratinizing squamous cell carcinomas of the lungs (highly significant) in females (tumor incidences reported in Tables 17, 18, and 19).

TABLE 17. HEPATOCELLULAR CARCINOMAS AND HEPATOCELLULAR HYPERPLASTIC NODULES IN FEMALE SPRAGUE-DAWLEY RATS MAINTAINED ON DIETS CONTAINING TCDD

Dose level ug/kg/day	Rats with hepatocellular hyperplastic nodules	Rats with hepatocellular carcinomas[a]	Total number of rats with both types of tumors[a]
0	8/86 (9%)	1/86 (1%)	9/86 (10%)
0.001 (22 ppt)	3/50 (6%)	0/50 (0%)	3/50 (6%)
0.01 (210 ppt)	18/50 (36%)	2/50 (4%)	18/50 (36%)[b] ($P = 4.37 \times 10^{-4}$)
0.1 (2200 ppt)	23/48 (48%)	11/48 (23%) ($P = 5.6 \times 10^{-5}$)	34/48 (71%) ($P = 9.53 \times 10^{-13}$)

[a]P-values calculated using the Fisher Exact Test (one-tailed).

[b]Two rats had both hepatocellular carcinomas and hyperplastic nodules.

TABLE 18. TUMOR INCIDENCE IN FEMALE RATS FED DIETS CONTAINING TCDD

Dose level ug/kg/day	Stratified squamous cell carcinomas of hard palate or nasal turbinates	Keratinizing squamous cell carcinomas of lungs
0	1/54 (2%)	0/86 (0%)
0.001 (22 ppt)	0/30 (0%)	0/50 (0%)
0.01 (210 ppt)	1/27 (4%)	0/49 (0%)
0.1 (2200 ppt)	5/24 (21%) ($P = 0.01$)[a]	7/49 (14%) ($P = 0.0006$)[a]

[a]P-values calculated using the Fisher Exact Test (one-tailed).

TABLE 19. TUMOR INCIDENCE IN MALE RATS FED DIETS CONTAINING TCDD

Dose level ug/kg/day	Stratified squamous cell carcinomas of the tongue		Hard palate/nasal turbinates stratified squamous cell carcinoma[a]	
0	0/76 (0%)		0/51 (0%)	
0.001 (22 ppt)	1/49 (2%)	N.S.[b]	1/34 (3%)	N.S.[b]
0.01 (210 ppt)	1/50 (2%)	N.S.[b]	0/27 (0%)	N.S.[b]
0.1 (2200 ppt)	3/42	($P = 4.3 \times 10^{-2}$)	4/30 (13%)	($P = 0.016$)

[a]Include examinations from both original and updated report (2/20/79).

[b]N.S. = not significant at $P = 0.05$.

Dr. Robert Squire, pathologist at the Johns Hopkins University Medical School and consultant to the CAG, evluated the histopathological slides from Dow Chemical Company's 2-year rat feeding studies on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by Kociba et al. Dr. Squire and his associates examined all livers, tongues, hard palates, and nasal turbinates, and lungs available from TCDD study. His histopathological findings, as well as Dr. Kociba's histopathological evaluations, are summarized in Tables 20 and 21 and Appendix B. Although there are some differences between the diagnoses of Kociba and Squire, the conclusions about the target organ for cancer induction, and the dose levels at which induction occurred are the same whether Squire's or Kociba's diagnoses are considered.

TABLE 20. DRS. SQUIRE'S AND KOCIBA'S REVIEW OF DOW TCDD ORAL RAT STUDY (8/15/80)
Sprague-Dawley Rats - Spartan Substrain (2 yrs.)

FEMALES

Tissues and Diagnoses	Dose Levels (ug/kg/day)							
	0 (control)		0.001		0.01		0.1	
	S	K	S	K	S	K	S	K
Lung Squamous cell carcinoma	0/86	0/86	0/50	0/50	0/49	0/49	8/47 ($P = 1.61 \times 10^{-4}$)	7/49 ($P = 1.21 \times 10^{-4}$)
Nasal turbinate/hard palate squamous cell carcinoma	0/54	1/54	0/30	0/30	1/27	1/27	5/22 ($P = 1.43 \times 10^{-3}$)	5/24 ($P = 9.53 \times 10^{13}$)
Liver Neoplastic nodules/ hepatocellular carcinoma	16/86	9/86	8/50	3/50	27/50 ($P = 2.42 \times 10^{-5}$)	18/50 ($P = 4.37 \times 10^{-4}$)	33/47 ($P = 4.92 \times 10^{-9}$)	34/48 ($P = 9.53 \times 10^{-1}$
Total combined (1, 2, or above)(each animal had at least one tumor above)	16/86	9/86	8/50	3/50	27/50 ($P = 2.42 \times 10^{-5}$)	18/50 ($P = 4.37 \times 10^{-4}$)	34/47 ($P = 1.20 \times 10^{-9}$)	34/39 ($P = 2.13 \times 10^{-1}$

S = Dr. Squire's histopathologic analysis
K = Dr. Kociba's histopathologic analysis

TABLE 21. DRS. SQUIRE'S AND KOCIBA'S REVIEW OF DOW TCDD ORAL RAT STUDY (8/15/80)
Sprague-Dawley Rats - Spartan Substrain (2 yrs.)

MALES

Tissues and Diagnoses	Dose Levels (ug/kg/day)							
	0 (control)		0.001		0.01		0.1	
	S	K	S	K	S	K	S	K
Nasal Turbinates/Hard Palate squamous cell carcinomas	0/55	0/51	1/34	1/34	0/26	0/27	6/30 ($P = 1.36 \times 10^{-3}$)	4/30?
Tongue Squamous cell carcinomas	0/77		2/44		1/49		3/44 ($P = 4.60 \times 10^{-2}$)	3/42 ($P = 4.34 \times 10^{-4}$)
Total - 1 or 2 above (each rat had at least one tumor above)	0/77		2/44		1/49		9/44 ($P = 6.28 \times 10^{-5}$)	

S = Dr. Squire's histopathologic analysis
K = Dr. Kociba's histopathologic analysis

National Cancer Institute (Oral) Rat Study (1980a, b)

A cancer bioassay for the possible carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was tested by the Illinois Institute of Technology in rats and mice under a contract sponsored by the National Cancer Institute (NCI).

In the rat study, 50 Osborne-Mendel rats of each sex were administered TCDD* suspended in vehicle of 9:1 corn oil-acetone by gavage 2 days per week for 104 weeks at doses of 0.01, 0.05, or 0.5 mg/kg/wk. Seventy-five rats of each sex served as vehicle controls. One untreated control group containing 25 rats of each sex was present in the TCDD treatment room and one untreated control group containing 25 rats of each sex was present in the vehicle control room. All surviving rats were killed at 105 to 107 weeks.

In rats, a dose-related depression in mean body weight gain became evident in the males after week 55 of the bioassay and in the females after week 45.

The results of histopathologic diagnosis of primary tumors caused by the oral administration of TCDD are presented in Table 22. In male rats an increased incidence of follicular-cell adenomas or carcinomas of the thyroid were dose-related and were statistically significantly higher in the low, mid, and high dose groups than in the vehicle controls. In addition, a statistically significant increase in subcutaneous tissue fibromas was found in males of the high dose group.

*Purity of TCDD was found to be 99.4%; two impurities tentatively identified as a trichlorodibenzo-p-dioxin and a pentachlorodibenzo-p-dioxin presence of 0.1 to 0.2% hexachlorodibenzo-p-dioxin was detected by gas chromatography and mass spectometry.

TABLE 22. INCIDENCE OF PRIMARY TUMORS IN MALE RATS ADMINISTERED TCDD BY GAVAGE

Type of tumor	Vehicle control	Low Dose[a] 0.01 ug/kg/week	Mid Dose[a] 0.05 ug/kg/week	High Dose[a] 0.5 ug/kg/week
Subcutaneous tissue Fibrosarcoma	3/75 (4%)	1/50 (2%)	3/50 (6%)	7/50 (14%) P = 0.048
Liver Neoplastic nodule or hepatocellular carcinoma	0/74 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adrenal Cortical adenoma	6/72 (8%)	9/50 (18%)	12/49 (24%)	9/49 (18%)
Thyroid Follicular cell adenoma	1/69 (1%)	5/48 (10%) P = 0.042	6/50 (16%) P = 0.021	10/50 (20%) P = 0.001
Thyroid Follicular cell adenoma or carcinoma	1/69 (2%)	5/48 (10%) P = 0.042	8/50 (16%) P = 0.004	11/50 (22%) P < 0.001

[a]P-values calculated using the Fisher Exact Test.

In female rats, a statistically significant increase of each of the following tumors was found in the high dose group: hepatocellular carcinomas and neoplastic nodules (P = 0.001), subcutaneous tissue fibrosarcomas (P = 0.023), and adrenal cortical adenomas (P = 0.039) as shown in Table 23.

These results confirm the carcinogenic effect observed in the Kociba et al. (1978) study using Sprague-Dawley (Spartan substrain) rats.

TABLE 23. INCIDENCE OF PRIMARY TUMORS IN FEMALE RATS ADMINISTERED TCDD BY GAVAGE

Type of tumor	Vehicle control	Low dose[a] 0.01	Mid dose 0.05	High dose[a] 0.5
			ug/kg/week	
Subcutaneous tissue Fibrosarcoma	0/75 (0%)	2/50 (4%)	3/50 (6%)	4/49 (8%) P = 0.023
Liver Neoplastic nodule	5/75 (7%)	1/49 (2%)	3/50 (6%)	12/49 (24%) P = 0.006
Liver Neoplastic nodule or hepatocellular carcinoma	5/75 (7%)	1/49 (2%)	3/50 (6%)	14/49 (29%) P = 0.001
Pituitary Adenoma	1/66 (2%)	5/47 (11%) P = 0.044	2/44 (5%)	3/43 (7%)
Adrenal Cortical adenoma	11/73 (15%)	8/49 (16%)	4/49 (8%)	14/46 (30%) P = 0.039

[a]P-values calculated using the Fisher Exact Test.

Van Miller et al. (Oral) Rat Study (1977)

Male Sprague-Dawley rats weighing approximately 60 grams each were used. There were 2 rats in each cage and 10 rats in each group. Rats ingested ground chow for only 2 weeks. They were then given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the following concentrations: 0, 1, 5, 50, 500 parts per trillion (ppt, 10^{-12} gram TCDD/gram food); and 1, 5, 50, 500, and 1000 parts per billion (ppb, 10^{-9} gram TCDD/gram food). Rats ingested the diets with TCDD for 78 weeks, and thereafter were kept on a control diet. Laparotomies were performed on all surviving rats at the 65th week and biopsies were taken from all tumors observed. Surviving rats were killed at 95 weeks.

Food intake was significantly lower in rats ingesting 50, 500, or 1000 ppb TCDD than in the controls, and they lost weight. All of the rats in the dose groups died between the second and fourth weeks of treatment. The food intake for rats receiving the other dose levels was similar to that of the controls. Weight gain was significantly less for rats given 5 ppb TCDD. TCDD intake and mortality of rats are shown in Table 24.

TABLE 24. TCDD INTAKE AND MORTALITY IN RATS

Dose[a]	Weekly dose per rat (ug/kg body weight)	Week of first death	Number of rats dead at 95th week
0 ppt	----	68	6/10 (60%)
1 ppt	0.0003	86	2/10 (20%)
5 ppt	0.001	33	4/10 (40%)
50 ppt	0.01	69	4/10 (40%)
500 ppt	0.1	17	5/10 (50%)
1 ppb	0.4	31	10/10 (100%)
5 ppb	2.0	31	10/10 (100%)

[a]Rats at 50, 500, and 1000 ppb dose levels were all dead within four weeks.

Complete necropsies were done and samples of tissues were taken for microscopic examination from the control groups and each treatment group (Laboratory audit* and personal communication with author). Special staining methods were used as an aid in the diagnosis of neoplasms. Various benign and malignant tumors were found in each treatment group. No tumors were observed in the controls (Table 25).

Statistically significant increases of squamous cell tumors of the lungs and neoplastic nodules of the liver were observed in rats ingesting 5 ppb TCDD (Table 26). In addition, two animals in the 5 ppb dose group and one animal in the 1 ppb dose group had liver cholangiocarcinomas, which are rare in Sprague-Dawley rats. These tumors were not found in any of the lower dose groups or in the controls. These results provide evidence of a carcinogenic effect.

The observation of no tumors of any kind in the controls is unusual for Sprague-Dawley rats. In addition, the reporting of the study was not extensive. These factors may tend to lessen the reliance which can be placed on the positive results of this study. However, this study does provide independent confirmation of the findings of the Kociba study that TCDD causes observable carcinogenic effects in rats at very low doses.

*The audit of this study brought out the fact that it was intended only to be a rangefinding study. Therefore, only small numbers of animals were used. This may have made the study relatively insensitive for detecting carcinogenic effects at doses lower than 1 ppb.

TABLE 25. BENIGN AND MALIGNANT TUMORS IN RATS INGESTING TCDD

Dose[a]	Benign	Malignant	Number of tumors	Number of rats with tumors
0	0	0	0	0/10 (0%)[b]
1 ppt	0	0	0	0/10 (0%)
5 ppt	1	5	6[c]	5/10 (50%)[d]
50 ppt	2	1	3[e]	3/10 (30%)
500 ppt	2	2	4[f]	4/10 (40%)[g]
1 ppb	0	4	5[h]	4/10 (40%)
5 ppb	8	2	10[i]	7/10 (70%)

[a]Rats at dose levels 50, 500, and 1000 ppb were all dead within four weeks.

[b]40 male rats used as controls for another study, received at the same time and kept under identical conditions, did not have neoplasms when killed at 18 months.

[c]1 rat had ear duct carcinoma and lymphocytic leukemia
1 adenocarcinoma (kidney)
1 malignant histiocytoma (retroperitoneal)
1 angiosarcoma (skin)
1 Leydig cell adenoma (testis)

[d]Three rats died with aplastic anemia.

[e]1 fibrosarcoma (muscle)
1 squamous cell tumor (skin)
1 astrocytoma (brain)

[f]1 fibroma (striated muscle)
1 carcinoma (skin)
1 adenocarcinoma (kidney)
1 sclerosing seminoma (testis)

[g]One rat had a severe liver infarction.

[h]1 rat cholangiocarcinoma and malignant histiocytomas (retroperitoneal)
1 angiosarcoma (skin)
1 glioblastoma (brain)
1 malignant histiocytoma (retroperitoneal)

[i]1 rat had squamous cell tumor (lung) and neoplastic nodule (liver)
2 cholangiocarcinoma and neoplastic nodules (liver)
3 squamous cell tumors (lung)
1 neoplastic nodule

TABLE 26. LIVER TUMORS IN RATS INGESTING TCDD

Dose(ppb)	Neoplastic nodules	Cholangio-carcinomas	Squamous cell tumors of the lungs
0	0/10 (0%)	0/10 (0%)	0/10
1	0/10 (0%)	1/10 (10%)	0/10
5	4/10 (20%) P = 0.043	2/10 (20%)[a]	4/10 (40%) P = 0.043

[a]The two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

Toth et al. (Oral) Mouse Study (1979)

This study investigated the carcinogenicity of TCDD in Swiss mice. Ten-week-old outbred Swiss /H/Riop mice were used. TCDD was administered in a sunflower oil vehicle by gavage to groups of 45 male mice once a week at doses of 7.0, 0.7, and 0.007 ug/kg body weight for a year (groups 9, 10, and 11, respectively, in Table 27). Matched male vehicle controls were administered sunflower oil once a week. Matched controls to a companion study investigating the carcinogenicity of (2,4,5-trichlorophenoxy)ethanol (TCPE) contaminated with low levels of TCDD, were administered carboxymethyl cellulose (the vehicle used in that study) once a week. Two untreated controls were also maintained.

This study appears to be generally well-conducted. However, the administration of TCDD over a period of only one year, which is far short of the life expectancy of the mice used, made the study relatively insensitive. Animals were followed for their entire lifetimes. Autopsies were performed after spontaneous death or when the mice were moribund, and all organs were examined histologically. Sections were stained with hematoxylin and eosin for light microscopy. Pathological findings were evaluated and analyzed statistically. The findings of the TCDD study and the comparison study on TCPE are given in Table 27 (reproduced from the journal in which this study is reported).

Analysis of the results of this study focused on the incidence of liver tumors in the groups treated with TCDD and the incidence of these tumors in the matched controls (group 12) and in the males in the three other control groups. Males in groups 3 and 8, the two untreated control groups, had 26% and 33% liver tumors, respectively ($P > 0.20$). The carboxymethyl cellulose male controls (group 7) had 33% (32/96) liver tumors. No significant differences in liver tumors were observed when males in all four control groups were compared to each

TABLE 27. CUMULATIVE DATA ON TUMOUR INCIDENCE
(Taken from Toth et al. 1979)

Group	Treatment				Effective no. of mice	No. of tumour bearing mice	Animals with tumours of				
	TCPE (mg/kg)	TCDD Vehicle[a] (ug/kg)	(mg/kg)	Sex			liver no.	(%)	lung no.	lymphomas no.	other organs no.	Average lifespan
1	67.0	0.112 (1.6 ppm)	50	M	88	69	42[b]	(18)	50	7	16	595
				F	83	61	7	(8)	52	15	25	652
2	70.0	0.007 (0.1 ppm)	50	M	98	78	57[c]	(58)	18	11	16	571
				F	96	59	9	(9)	39	15	23	582
3		control		M	93	63	24	(26)	44	8	17	577
				F	84	57	4	(5)	41	23	13	639
4	7.0	0.07 (10 ppm)	50	M	93	79	25	(27)	38	18	22	641
				F	96	60	10	(10)	38	19	19	589
5	7.0	0.0007 (0.1 ppm)	50	M	94	77	23	(24)	50	23	17	660
				F	93	71	8	(9)	42	36	21	590
6	0.7	0.00007 (0.1 ppm)	50	M	97	78	24	(25)	51	20	17	643
				F	94	64	5	(5)	38	22	21	566
7	--	--	50	M	96	74	32	(33)	44	14	22	615
				F	84	55	4	(5)	38	18	17	565
8		control		M	96	78	32	(33)	38	22	15	651
				F	91	57	4	(4)	31	24	19	549
9		7.0	10	M	43	27	13	(30)	11	6	7	424
10		0.7	10	M	44	36	21	(48)	18	12	4	633
11		0.007	10	M	44	39	13	(29)	27	10	6	649
12	--	--	10	M	38	27	7	(18)	15	6	7	588

[a]Carboxymethyl cellulose in groups 1-8, sunflower oil in groups 9-12.

[b]$p < 1\%$

[c]$p < 0.1\%$

other ($P > 0.05$). Nevertheless, there was evidence that the incidence of liver tumors in the control groups was associated with the average lifespan in the respective groups. The two groups that had less than 600 days average survival (groups 3 and 12) had the fewest liver tumors (26% and 18%, respectively). On the other hand, the two groups that had an average survival of greater than 600 days (groups 7 and 8), had 33% liver tumors each. The test for linear trend (tumors vs. days of average survival) was not quite significant ($P = 0.065$).

Among the three treatment groups (groups 9, 10, and 11), the middle dose (0.7 ug/kg) showed the highest incidence of liver tumors (21/44 = 48%). This incidence was significantly higher than the incidence of liver tumors in either the sunflower oil controls ($P < 0.01$) or the pooled controls (all four control groups combined) ($P < 0.025$).

The highest dose group (7.0 ug/kg) had an increased incidence of liver tumors compared to the matched sunflower oil controls (13/43 = 30%) but this increase was not statistically significant ($P = 0.11$). The incidence of liver tumors in the high dose group was comparable to that of the pooled controls. The highest dose group, however, had a much reduced average survival in comparison to any of the control groups (only 424 days compared to 577, 588, 615, and 651 days in the four control groups). This poor survival may have accounted for the lack of a statistically significant increase in liver tumors in the high dose group. Furthermore, if time-to-tumor data had been available, it is highly likely that the high dose group would have shown a significant decrease in time-to-tumor compared to the controls. Therefore, the increase in liver tumors that was observed in the high dose group in comparison to the matched control group, although not statistically significant, is considered to be consistent with an oncogenic effect.

In conclusion, the results of this study provide suggestive evidence of an oncogenic effect.

National Cancer Institute (Oral) Mouse Study (1980a, b)

A cancer bioassay for the possible carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin was tested by the Illinois Institute of Technology in mice under a contract sponsored by the National Cancer Institute (NCI).

In the mouse study, 50 B6C3F1 mice of each sex were administered TCDD suspended in a vehicle of 9:1 corn oil-acetone 2 days per week for 104 weeks at doses of 0.01, 0.05, and 0.5 ug/kg/wk for male mice and 0.04, 0.2, and 2.0 ug/kg/wk for female mice. Seventy-five mice of each sex were used as vehicle controls. One untreated control group of 25 mice of each sex was present in the TCDD treatment room. One untreated control group of 25 mice of each sex was present in the vehicle control room. In mice, the mean body weight gain in the treated groups was comparable with that of the vehicle control groups. However, the mean body weight of the treated mice was lower when it was compared with untreated controls.

The results of the histopathologic diagnosis of primary tumors are presented in Table 28. The results indicate that, in male mice, TCDD induced a statistically significant incidence of hepatocellular carcinomas ($P = 0.002$) and both hepatocellular carcinomas and neoplastic nodules combined ($P = < 0.001$) in male mice of the high dose group.

In female mice, TCDD induced statistically significant increases of hepatocellular carcinomas ($P = 0.014$) and both hepatocellular adenomas and carcinomas ($P = 0.002$) in the high dose group. In addition, a statistically significant increase in tumor incidences of fibrosarcoma, histiocytic lymphoma,

thyroid follicular-cell adenoma, and cortical adenoma or carcinoma were also observed in the high dose group (Table 29).

The incidence of liver tumors observed in this study confirms the earlier observation of an increase in liver tumors in the male mouse study performed by Toth et al. (1979).

TABLE 28. INCIDENCE OF PRIMARY TUMORS IN MALE MICE ADMINISTERED TCDD BY GAVAGE

Type of tumor	Vehicle control	Low dose 0.01	Mid dose 0.05	High dose[a] 0.5
			ug/kg/week	
Liver Hepatocellular adenoma	7/73 (10%)	3/49 (6%)	5/49 (10%)	10/50 (20%)
Liver Hepatocellular carcinomas	8/73 (11%)	9/49 (18%)	8/49 (16%)	17/50 (34%) P = 0.002
Liver Hepatocellular adenoma and carcinomas	15/73 (21%)	12/49 (24%)	13/49 (27%)	27/50 (54%) P < 0.001

[a]P-values calculated using the Fisher Exact Test.

TABLE 29. INCIDENCE OF PRIMARY TUMORS IN FEMALE MICE ADMINISTERED TCDD BY GAVAGE

Type of tumor	Vehicle control	Low dose 0.04	Mid dose 0.2	High dose[a] 2.0
			ug/kg/week	
Subcutaneous tissue Fibrosarcoma	1/74 (1%)	1/50 (2%)	1/48 (2%)	5/47 (11%) P = 0.032
Hematopoietic system Histiocytic lymphoma	9/74 (12%)	4/50 (8%)	4/48 (17%)	14/47 (30%) P = 0.016
Hematopoietic system Lymphoma or leukemia	18/74 (24%)	11/50 (22%)	13/48 (27%)	20/47 (43%) P = 0.029
Hematopoietic system All lymphoma	18/74 (24%)	12/50 (24%)	13/48 (27%)	20/47 (43%) P = 0.029
Liver Hepatocellular carcinomas	1/73 (1%)	2/50 (4%)	2/48 (4%)	6/47 (13%)
Liver Hepatocellular adenomas or carcinomas	3/73 (4%)	6/50 (12%)	6/48 (13%)	11/47 (23%) P = 0.002
Thyroid Follicular-cell adenoma	0/69 (0%)	3/50 (6%)	1/47 (2%)	5/46 (11%) P = 0.009

[a]P-values calculated using the Fisher Exact Test.

Other Related Studies

Pitot et al. Promotion Study in Rats (1980) --

Pitot et al. (1980) investigated the hypothesis that development of hepatocellular carcinomas of the liver with chronic administration of TCDD was the result of the promoting activity of TCDD on cells already initiated by dietary or other environmental carcinogens. The manuscript of this study has been submitted to Cancer Research for publication.

In this study, a two-stage model of hepatocarcinogenesis was used. Twenty-four hours after a partial hepatectomy (to cause cell proliferation), female Sprague-Dawley rats were divided into seven groups (Table 30). The animals in groups 1, 5, 6, and 7 received diethylnitrosamine (DEN). The rats in group 1 were then maintained on a standard laboratory diet for 32 weeks. The rats in groups 2 and 3 received no DEN, but starting one week after hepatectomy received biweekly subcutaneous injections of 0.14 or 1.4 ug/kg of TCDD in corn oil for a period of 28 weeks (TCDD was 98.6% pure and provided by Dow Chemical Co.). Groups 5 and 6 received DEN, and one week later were initiated on a regimen of 14 biweekly injections of 0.14 and 1.4 ug/kg of TCDD. The animals in group 4 received 0.05% sodium phenobarbital in the diet starting one week after partial hepatectomy for 28 weeks, and the animals in group 5 received DEN and one week later were also administered 0.05% sodium phenobarbital in the diet for the duration of the experiment. At the end of the experiment, rats were killed and sections of the liver were removed and frozen on solid CO_2. Serial sections of the frozen blocks of liver were cut and stained consecutively for glucose-6-phosphatase (G6Pase), canalicular ATPase, γ-glutamyl transpeptidase GGTase) with haematoxylin and eosin. The number of enzyme-altered foci were determined from photographs of histochemically stained sections. Hepatocarcinomas were diagnosed by standard histopathological criteria.

TABLE 30. PROMOTING EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON HEPATOCARCINOGENESIS BY A SINGLE DOSE OF DIETHYLNITROSAMINE (DEN) AND PARTIAL HEPATECTOMY (PH)[a]

Group Number	Treatment	n[b]	Number of enzyme-altered foci per cm^3 of liver	Percent liver volume which is enzyme-altered foci	Number of rats with carcinoma
1	PH + DEN	(4)	346 ± 65	5.0	0
2	PH + TCDD (low dose)	(5)	46 ± 15	0.1	0
3	PH + TCDD (high dose)	(5)	76 ± 20	0.1	0
4	PH + Phenobarbital	(6)	138 ± 40	0.1	0
5	PH + DEN + TCDD (low dose)	(5)	1582 ± 300	7.8	0[c]
6	PH + DEN + TCDD (high dose)	(7)	1280 ± 40	35.0	5/7[d] (P = 0.0075)
7	PH + DEN + Phenobarbital	(4)	1510 ± 185	5.0	2

[a]Female rats (200 g) were intubated where shown with DEN. Seven days later TCDD (injected subcutaneously) or phenobarbital (0.05% in the diet) administration was begun and continued for 28 weeks at which time the animals were sacrificed and the livers examined. The low and high doses of TCDD were 0.14 and 1.4 ug/kg/2 weeks, respectively, administered subcutaneously. DEN was given at a dose of 10 mg/kg. See text for further details.

[b]The numbers in parentheses denote the number of animals used in each group.

[c]Three rats showed "neoplastic nodules."

[d]One rat showed a "neoplastic nodule."

[e]P-value calculated using the Fisher Exact Test.

The results presented in Table 30 showed that the number of foci with single enzyme changes, the number of foci with multiple enzyme changes, and the total liver volume affected, substantially increased with the administration of TCDD. No carcinomas were detected in four rats treated with DEN only, but five of seven rats treated biweekly with TCDD at 1.4 ug/kg in addition to DEN had hepatocellular carcinomas, and six of seven rats had hepatocellular carcinomas or hepatocellular neoplastic nodules with a statistical significance ($P = 0.0075$). Three of five rats treated biweekly with TCDD at 0.14 ug/kg in addition to DEN had hepatocellular neoplastic nodules ($P = 0.083$). Rats receiving only TCDD after partial hepatectomy showed no significant increase in enzyme-altered foci and no neoplasia.

The results of this study provide evidence that TCDD acts as a potent promoter in this two-stage model of hepatocarcinogenesis, causing increased neoplasia and increases in enzyme-altered foci at exceedingly low levels.

National Cancer Institute Skin Painting Study in Mice (1980b) --

This cancer bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for possible carcinogenicity was tested by the Illinois Institute of Technology under a contract sponsored by the National Cancer Institute (NCI) in Swiss-Webster mice. In this study, groups of 30 male and female Swiss-Webster mice were used. TCDD in acetone suspension was applied to skin of mice 3 days per week for 104 weeks. Male mice received 0.001 ug TCDD per application while the female mice received 0.005 ug TCDD per application.

In another experiment, the same number of animals were pretreated with one application of 50 ug 7,12-dimethylbenz(a)anthracene (DMBA*) in 0.1 ml acetone

*DMBA obtained from K and K Laboratories (Cleveland, Ohio). Its purity was not evaluated by NCI but stated by the manufacturer to be at least 95%.

one week before TCDD application was initiated. Forty-five mice of each sex received 0.1 ml acetone three times per week and 30 animals of each sex were used as untreated controls; no DMBA control was used.

Mean body weights of male and female groups of mice treated with TCDD or TCDD following a single application of DMBA were not affected as compared to the vehicle controls. Mean body weights of treated and vehicle control groups of females were lower than those of untreated controls. Mean body weights of males were less than mean body weights of untreated controls.

The results of histopathologic diagnosis are shown in Table 31. The results show that TCDD induced statistically significant ($P < 0.05$) increases of fibrosarcoma in the integumentary systems of female mice treated with TCDD alone and TCDD following a single initial application of DMBA.

TABLE 31. INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED TCDD OR TCDD FOLLOWING DMBA BY DERMAL APPLICATION

Type of tumors	Dose levels		
	Vehicle Control	TCDD[a]	DMBA (50) ug) plus TCDD[a]
		MALE	
Integumentary system		0.001 ug x 3/wk	0.001 ug x 3/wk
Fibrosarcoma	3/42 (7%)	6/28 (21%) P = 0.08	6/30 (20%) P = 0.10
		FEMALE	
		0.005 ug x 3/wk	0.005 ug x 3/wk
Fibrosarcoma	2/41 (5%)	8/27 (30%) P = 0.007	8/29 (28%) P = 0.010

[a]P-value calculated using the Fisher Exact Test.

Berry et al. Skin Painting Study in Mice (1978, 1979) --

Berry et al. (1978) applied TCDD in acetone solution at 0.1 ug/mouse twice weekly for 30 weeks to the skin of 30 female Charles River CD-1 mice after initiation with a single dermal application of the known skin carcinogen DMBA in acetone. After 30 weeks of promotion with TCDD, no papillomas were observed on the DMBA-initiated mice. In the positive controls, DMBA-initiated mice were treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) for 30 weeks; 92% of these mice developed tumors.

Berry et al. (1979) also studied the effects of treatment with TCDD and 7,12-dimethylbenz(a)anthracene (DMBA) in a two-stage tumorigenesis bioassay in mouse skin. In this study, tumors on the shaved skin of female CD-1 mice were initiated by topical application of DMBA and were promoted with TPA. Pretreatment with TCDD markedly inhibited the initiation of tumors by DMBA. The effects were greatest when TCDD was applied 3 to 5 days before initiation and were negligible when it was applied only 5 minutes before initiation. The inhibition was almost complete (94 to 96%) when a single dose of 1 ug of TCDD/mouse was applied, but was only slightly less effective (89%) when the dose was reduced to 10 ug/mouse. The time course of the inhibitory effects was closely parallel to the time course of induction of arylhydrocarbon hydroxylase in the skin of the mice. It was also associated with substantial reduction in the covalent binding of the DMBA metabolite to DNA and RNA, but with no change in their binding to protein.

The same authors also reported inhibitory effects of TCDD on the initiation of mouse skin tumors by benz(a)pyrene (BAP), although the effect was not as large (maximum 65%) with BAP as with DMBA.

Cohen Skin Painting Study in Mice (1979) --

Cohen et al. (1979) showed that pretreatment of mice with dermally applied TCDD resulted in the inhibition of skin tumor induction by subsequent treatment with DMBA and BAP. The inhibition of skin carcinogenesis by BAP in mice after pretreatment with TCDD was associated with an increase in covalent binding of BAP metabolites to DNA, RNA, and protein (in contrast to the results with DMBA, which showed a reduction in binding to DNA and RNA). However, the BAP metabolites that were bound to DNA and RNA in mice pretreated with TCDD differed from those in untreated mice. In particular, pretreatment with TCDD markedly reduced the formation of the presumptive ultimate carcinogenic metabolite of BAP, 7,8-diol-9,10-epoxy BAP, and its covalent binding with guanosine in DNA.

Kouri et al. Mouse Study (1978) --

This study was designed as an investigation of the cocarcinogenic activity of TCDD administered to mice in conjunction with subcutaneous administration of 3-methylcholanthrene (MCA). Two inbred strains of mice, C57BL/6Cum (abbreviated B6) and DBA/2Cum (abbreviated D2), were used. These strains are responsive and nonresponsive, respectively, to the induction of aryl hydrocarbon hydroxylase (AHH) by MCA.

Groups of mice of both sexes were injected subcutaneously at 4 to 6 weeks of age with either 150 ug of MCA dissolved in trioctanoin or with trioctanoin alone. Some groups were also injected with TCDD dissolved in p-dioxane, either simultaneously with the administration of MCA or 2 days earlier. Two doses of TCDD (1 ug/kg and 100 ug/kg) were used, and the effects of both intraperitoneal and subcutaneous injections were investigated. Two sets of experiments, involving 29 groups of mice, were conducted approximately 1 year apart (Tables 32 and 33).

After treatment, the mice were observed for 36 weeks, during which time they were palpated weekly for the presence of tumors; latency was calculated when the subcutaneous tumors became 1 cm in diameter. Only tumors characterized histologically as fibrosarcomas at the site of inoculation were considered. It is unclear whether or not these were the only tumor types observed. The term "carcinogenic index" used by the authors was defined as the percentage of tumor incidence 8 months after treatment divided by the average latency in days multiplied by 100. No details were given of the number of animals in each group at the start of each experiment but the numbers dying in the first 28 days and the numbers at risk (surviving 36 weeks) were tabulated. The results of this study are shown in Tables 32 and 33.

No subcutaneous tumors were observed in controls or in mice treated with TCDD alone. In B6 (responsive) mice, the administration of TCDD did not significantly enhance the induction of tumors by MCA. However, in both experiments involving D2 (nonresponsive) mice, the administration of TCDD simultaneously with MCA appeared to enhance the carcinogenic response. The "carcinogenic index" increased from 1 to 6 in groups treated with MCA alone to 14 in the group treated subcutaneously with TCDD at 1 ug/kg, and 13 to 15 in the groups treated intraperitoneally with TCDD at 100 ug/kg. The authors concluded that TCDD acts as a cocarcinogen. They speculated that it may act by local induction of AHH at the site of inoculation.

A more appropriate statistical analysis would be a comparison of tumor incidence in TCDD-treated groups with tumor incidence in corresponding MCA-treated groups within the same experiment. The results of this analysis are given in Table 34.

From these results, the CAG concluded that the experiment adequately

TABLE 32. EFFECTS OF INTRAPERITONEAL ADMINISTRATION OF TCDD ON MCA-INITIATED SUBCUTANEOUS TUMORS (Kouri et al. 1978)

Inbred strain	Treatment		No. of mice dying because of treatment[a]	No. of mice at risk for tumors[b]	No. of mice with tumors[c]	% of mice with tumors	Average latency (days)	Carcinogenic index[d]
	-2 days	0 days						
B6	i.p. p-dioxin	s.c. trioctanoin	1	39	0	0		
	i.p. TCDD (100 ug/kg)	s.c. trioctanoin	20	27	0	0		
	None	s.c. MCA	1	36	29	81	125	65
	None	i.p. TCDD (100 ug/kg)	20	30	0	0		
	None	i.p. TCDD (100 ug/kg) + s.c. MCA	30	43	33	71	123	63
	None	i.p. TCDD (1 ug/kg)	4	46	0	0		
	None	i.p. TCDD (1 ug/kg) + s.c. MCA	6	27	27	100	132	76
	i.p. TCDD (100 ug/kg)	s.c. MCA	20	25	21	84	129	65
	i.p. TCDD (1 ug/kg)	s.c. MCA	6	23	16	70	140	50
D2	i.p. p-dioxane	s.c. trioctanoin	6	22	0	0		
	i.p. TCDD (100 ug/kg)	s.c. trioctanoin	24	25	0	0		
	None	s.c. MCA	3	34	1	3	217	1
	None	i.p. TCDD (100 ug/kg)	30	38	0	0		
	None	i.p. TCDD (100 ug/kg) + s.c. MCA	43	43	10	23	178	13[e]
	None	i.p. TCDD (1 ug/kg)	5	48	0	0		
	None	i.p. TCDD (1 ug/kg) + s.c. MCA	5	34	5	15	199	7
	i.p. TCDD (100 ug/kg)	s.c. MCA	20	28	0	0		
	i.p. TCDD (1 ug/kg)	s.c. MCA	6	31	0	0		

[a]During the first 28 days following treatment

[b]Defined as the number of mice surviving the 36-week observation period.

[c]At the end of the 36-week experiment

[d]Percentage of incidence of tumors, divided by the average latency in days, multiplied by 100 (8).

[e]This carcinogenic index value lies outside (greater than) the 99% confidence interval (i.e. $P < 0.01$) constructed from seven different studies over the past 5 years during which 150 ug of MCA was given s.c. to D2 mice. These studies included 295 D2 mice, the mean = 5 D for all seven studies was a carcinogenic index of 5.43 = 2.70.

TABLE 33. EFFECT OF INTRAPERITONEAL OR SUBCUTANEOUS ADMINISTRATION OF TCDD GIVEN 2 DAYS BEFORE OR SIMULTANEOUS WITH SUBCUTANEOUS ADMINISTRATION OF MCA ON TUMORIGENESIS IN D2 MICE
(Kouri et al. 1978)

Treatment		No. of mice dying because of treatment	No. of mice at risk for tumors	No. of mice with tumors	% of mice with tumors	Average latency (days)	Carcinogenic index
-2 days	0 days						
None	s.c. MCA	0	30	3	10	177	6
i.p. p-dioxane	s.c. MCA	10	40	4	10	194	5
i.p. TCDD (100 ug/kg)	s.c. MCA	35	65	9	14	145	10
None	i.p. p-dioxane x s.c. MCA	5	45	5	11	176	6
None	i.p. TCDD (100 ug/kg) + s.c. MCA	38	62	17	27	183	15[a]
None	i.p. TCDD (1 ug/kg) + s.c. MCA	22	78	8	10	162	6
None	s.c. p-dioxane + s.c. MCA	2	68	8	12	180	6
None	s.c. TCDD (100 ug/kg)	8	42	0	0		
None	s.c. TCDD (100 ug/kg + s.c. MCA	18	82	46	55	145	38[a]
None	s.c. TCDD (1 ug/kg)	2	48	0	0		
None	s.c. TCDD (1 ug/kg + s.c. MCA	2	98	21	21	154	14[a]

[a]These carcinogenic index values lie outside the 99% confidence interval.

TABLE 34. INCIDENCE OF TUMORS IN MICE TREATED WITH MCA AND WITH MCA AND TCDD

Experiment	Dose of TCDD	Route of administration	Tumor Incidence		P-value[b]
			TCDD and MCA	MCA	
1	100 ug/kg	Intraperitoneal	10/43	1/34[a]	P = 0.01
2	100 ug/kg	Intraperitoneal	17/62	5/45	P = 0.03
2	100 ug/kg	Subcutaneous	46/82	5/45	$P = 3.0 \times 10^{-7}$
2	1 ug/kg	Subcutaneous	21/98	5/45	P = 0.1

[a]Vehicle (p-dioxane) not administered.

[b]p-value calculated using the Fisher Exact Test (one-tailed).

demonstrated the enhancement by TCDD of tumor induction when TCDD was administered simultaneously with MCA at the higher dose (100 ug/kg). The reported results at the lower dose (1 ug/kg) are not statistically significant unless the reduction in latency is taken into account, which is difficult to do rigorously. Despite defects in reporting (failure to specify the initial number of animals in each group and to report tumor incidence by sex), the results provide convincing evidence that TCDD acts as a cocarcinogen. The failure of TCDD to induce tumors when administered alone was not unexpected since only a single dose was administered and the duration of the study was very short (36 weeks).

ESTIMATION OF TCDD LEVELS IN 2,4,5-T STUDIES

As discussed above, all of the 2,4,5-T studies which either did not demonstrate an oncogenic effect or had ambiguous results (i.e., all 2,4,5-T studies except the one conducted by Kociba), failed to provide significant evidence of either the oncogenicity or lack of oncogenicity of 2,4,5-T because of deficiencies in their design. However, it is useful to calculate the highest doses of TCDD which were administered in these studies as a contaminant of 2,4,5-T and compare these doses to the doses of TCDD which induced an oncogenic response in the TCDD studies. The calculated doses are based on the reported contamination levels of the 2,4,5-T used in the studies and the dose of 2,4,5-T administered. Tables 35 and 36 show that the doses of TCDD administered as a contaminant in each of the 2,4,5-T studies, with the possible exception of the Bionetics oral mouse study, were below those doses which produced an observable oncogenic response in the TCDD studies on the same species. Thus, especially in view of the deficiencies and insensitivity of the 2,4,5-T studies, it is not surprising that the TCDD contamination did not induce an observable oncogenic effect. In the Bionetics oral mouse study, if it is assumed that the 2,4,5-T administered was contaminated with 30 ppm TCDD, then the TCDD dose was 0.27 ug/kg/day. This is higher than or equal to the dose which induced an oncogenic response in the NCI oral mouse study (0.071 and 0.28 ug/kg/day in male and female mice, respectively). The absence of an oncogenic effect in the Bionetics study may be explained by several factors. First, the study was much more insensitive than either the Toth or NCI studies because the group sizes were much smaller. Second, different strains or substrains of mice were used. Third, as explained earlier, the TCDD contamination may not have been as high as 30 ppm.

TABLE 35. COMPARISON OF DOSE LEVELS OF TCDD IN 2,4,5-T[a] STUDIES WITH RESPECT TO THE TCDD STUDY IN MICE WHERE POSITIVE TUMOR INCIDENCE WAS OBSERVED

Study	Strain of mouse	Route	Dose-level 2,4,5-T mg/kg/day	Dose-level TCDD ug/kg/day	Tumors observed
(Innes) Bionetics	F_1 hybrid of C57bl/6 and C3H/AWf (Strain "A") or "X"	diet	9	0.27	-
	F_1 hybrid of C57Bl/6 and AKR (Strain "Y" or "B")	diet	9	0.27	-
Muranyi-Kovacs	XVIIG	diet	12	6.0×10^{-4}	-
	C3Hf	diet	12	6.0×10^{-4}	-
NCI	B6C3F1 Male[b]	gavage	--	1.42×10^{-3}	
				7.1×10^{-3}	
				7.1×10^{-2}	+
	B6C3F1 Female[b]	gavage	--	5.7×10^{-3}	
				2.85×10^{-2}	
				0.285	+
Toth	Swiss male	gavage	--	1.0	+
				0.1	+
				0.001	
(Innes) Bionetics	"A or Y"	subcutaneous	215 mg/kg (one dose only)	6.4 (one dose only)	-
	"Y or B"		--	--	-
Muranyi-Kovacs	$XVIIG_1$	subcutaneous	10(4 doses only)	5×10^{-4} (4 doses only)	
	C3Hf		10(4 doses only)	5×10^{-4} (4 doses only)	-

[a]TCDD contaminant in 2,4,5-T
30 ppm--Innes et al. Study (assumed in this analysis, see page 32)
0.05 ppm--Muranyi-Kovacs et al. Study
0.05 ppm--Leuschner et al. (German Study)
0.33 ppb--Dow Chemical Company Study

[b]Carcinogenic in male and/or female.

TABLE 36. COMPARISON OF DOSE LEVELS OF TCDD IN 2,4,5-T STUDIES WITH RESPECT TO THE TCDD STUDY IN RATS WHERE POSITIVE TUMOR INCIDENCE WAS OBSERVED

Study	Strain	Route	Dose-level 2,4,5-T mg/kg/day	Dose-level TCDD ug/kg/day	Tumors observed
Kociba 2,4,5,T[a]	Sprague-Dawley (Spartan)	diet	3	1.0×10^{-6}	-
			10	3.3×10^{-6}	-
			30	9.9×10^{-6}	+ (?)
Leuschner 2-4-5-T[b]	Sprague-Dawley (SIV50)	"	3	1.5×10^{-4}	-
			10	5×10^{-4}	-
			30	1.5×10^{-3}	?
Kociba TCDD	Sprague-Dawley (Spartan)	"	--	1×10^{-3}	-
			--	1×10^{-2}	+
			--	1×10^{-1}	+
NCI TCDD	Osborne-Mendel	gavage	--	1.42×10^{-3}	+
			--	7.1×10^{-3}	+
			--	7.1×10^{-2}	+
Van Miller TCDD[c]	Sprague-Dawley	diet	--	5.0×10^{-2}	+ (?)
			--	2.50×10^{-1}	+ (?)

[a]Marginally positive response ($P = 0.063$) for carcinoma of the tongue in the 30 mg/kg/day group, compared to matched controls using Kociba's diagnoses; significant ($P < 0.001$) when compared to historical controls; significant when using Squire's diagnoses ($P = 0.025$). Although no detectable TCDD was present, it is assumed for this analysis that TCDD is present at the level of detection (0.33 ppb).

[b]Significance of increase of interstitial cell tumors of testes in the 30 mg/kg/day group is unclear because of great disparity in incidences in two different control groups and because historical controls untreated with acetone had comparable incidences of these tumors.

[c]Certain aspects of this study tend to lessen the reliance which may be placed on its results.

Potency of TCDD

The carcinogenic potency of TCDD is greater than that of aflatoxin B_1, which is one of the most potent carcinogens known. This conclusion comes from a comparison of the tumor incidence in male Fischer rats (Wogan et al. 1974), which were fed 50 ppb of aflatoxin B_1, with the incidence of the same tumor type in female Sprague-Dawley rats (Kociba et al. 1977) fed 0.1 ug/kg/day (2.2 ppb). The potency of each of these compounds was estimated by calculating the slope of the linear one-hit model for these compounds. The slope (B) is calculated according to the following formula:

$$B = \frac{1}{d} \ln \frac{(1 - P_c)}{(1 - P_t)}$$

d = dose inducing carcinogenic effect in the respective studies on TCDD and aflatoxin.

P_c = tumor incidence in control animals in the respective studies.

P_t = tumor incidence in treated animals in the respective studies at dose d.

This calculation was made on the basis of the lowest dose level at which TCDD or aflatoxin B_1 caused a significant increase in hepatocellular carcinomas, the incidence of hepatocellular carcinomas at the respective dose levels, and the spontaneous incidence of this type of cancer in the control animals of each study.*

Table 37 shows that TCDD is more potent than aflatoxin by a factor of 0.110/0.032 = 3.45. On this basis, it is estimated that TCDD is a more potent carcinogen than aflatoxin B_1 roughly by a factor of three.

*Wogan et al. are not clear on their histologic classification of preneoplastic lesions. Therefore, only carcinomas were selected for calculating B.

TABLE 37. COMPARISON OF CARCINOGENIC POTENCY OF TCDD WITH AFLATOXIN B_1

	TCDD	Aflatoxin B_1
Author	Kociba et al. 1977	Wogan et al. 1974
Species	Sprague-Dawley rats	Fischer rats
Sex	Female	Male
Tumor incidence in controls (P_c)	1/86	0/18
Dose (d), Tumor incidence in treated animals (P_t)	2.2 ppb, 11/49	50 ppb, 20/25
Carcinogenic potency (B)	0.110 $(ppb)^{-1}$	0.032 $(ppb)^{-1}$

SUMMARY OF LABORATORY ANIMAL STUDIES ON 2,4,5-T, SILVEX, AND TCDD

There is highly suggestive evidence that 2,4,5-T is carcinogenic in rats. The chronic mouse studies on 2,4,5-T suffered from deficiencies in design or conduct which made them insensitive for detecting an oncogenic response. Therefore, these studies do not provide significant evidence of either the carcinogenicity or non-carcinogenicity of 2,4,5-T in mice.

All the chronic animal studies on silvex suffered from deficiencies in design or conduct which made them insensitive for the purpose of detecting an oncogenic effect. Therefore, these studies do not provide significant evidence of either the carcinogenicity or non-carcinogenicity of silvex.

Studies on rats and mice provide substantial evidence that TCDD is carcinogenic in rats and mice. There is also evidence that TCDD is a potent liver cancer promoter and is a cocarcinogen. On the basis of the Dow study (Kociba et al. 1977, 1978), it appears that TCDD is one of the most potent carcinogens known.

The question arises as to whether the carcinogenic action of TCDD by itself such as exhibited in the Kociba et al. and the N[illegible] studies on rats and mice could be due to the action of TCDD as a carcinogen and/or a promoting agent. There is evidence that TCDD can be metabolized to a reactive electrophilic metabolite which could react with DNA and thereby produce genetic damage of the sort that is associated with the induction of cancer. However, the reactivity of this metabolite is extremely high with cellular proteins and, to date, the degree of interaction with DNA that has been demonstrated is low. This may be peculiar to the tissues that have been examined for this reaction so far but may not be generally applicable to the reaction of TCDD with DNA in the body. Furthermore, TCDD has a chemical structure which makes it likely that it could intercalate into DNA and also act as a genotoxic carcinogen. Promoting agents, when administered alone, characteristically produce a relatively small increase in the occurrence of tumors and these tumors are of the sort that occur spontaneously. This is not characteristic of TCDD, particularly in relation to its ability to induce squamous carcinomas of the lung and of the hard palate and nasal turbinates. Squamous carcinomas of the lung are exceedingly uncommon in the rat in contrast to adenomas of the lung. For these reasons, the CAG believes that it is prudent, given the present state of knowledge, to regard TCDD as a complete carcinogen as well as a promoting and cocarcinogenic agent.

VI. EPIDEMIOLOGIC STUDIES

SOFT TISSUE SARCOMA

Prompted by clinical observations over a 7-year period of malignant sarcomas in seven men with previous occupational exposure to phenoxyacetic acid herbicides (Hardell 1977), researchers at the Department of Oncology, University Hospital, Umea, Sweden initiated epidemiologic studies to test the hypothesis of an etiologic association. The investigators elected to conduct case-control studies, a type of epidemiologic research particularly well suited for rare diseases with long periods of induction (Cole 1979). Cases were defined as male patients with sarcomas of soft connective tissue, such as smooth muscle (leiomyosarcoma) and fat (liposarcoma). The distributions of tumor types in the two studies are shown in Table 38. Sarcomas of harder connective tissues, such as bone and cartilage, were excluded because they were not present in the original series of cases.

TABLE 38. DISTRIBUTION OF TUMOR TYPES IN TWO CASE-CONTROL STUDIES OF SOFT TISSUE SARCOMA

Diagnosis	Tissue of origin	Percent of cases Study A (n=52)	Study B (n=110)
Leiomyosarcoma	Smooth muscle	30	23
Fibrous histiocytoma	Subcutaneous connective tissue	17	25
Liposarcoma	Fat tissue	14	6
Neurogenic sarcoma	Nerve tissue	10	4
Angiosarcoma	Blood vessels	8	2
Myxosarcoma	Primitive connective tissue	6	8
Fibrosarcoma	Fibrous tissue	4	8
Other sarcomas		11	24
Total		100	100

Sources: Study A, unpublished information supplied by Hardell to EPA.
Study B, Eriksson et al. (1979)

Two case-control studies were conducted, the first in northern Sweden (referred to below as Study A), and the second in the southern part of the country (Study B). The frequencies of exposure to the substances of primary interest are shown in Table 39. In the north, occupational exposure to phenoxyacetic acids took place in both forestry and agricultural work. In the south, these exposures were predominantly agricultural. The phenoxyacetic acids to which exposure occurred consisted predominantly of 2,4,5-T and 2,4-D in both studies. Exposure to 2,4,5-T in the absence of 2,4-D was rarely reported in either study. Exposure to chlorophenols, which contain chlorinated dibenzodioxin impurities (Levin et al. 1976), occurred mostly in sawmill work and paper pulp production. Very few persons reported joint exposure to both phenoxyacetic acids and chlorophenols in these studies.

Of the two phenoxyacetic acids to which exposure predominantly occurred (2,4,5-T and 2,4-D), only 2,4,5-T is known to be contaminated with TCDD. There are two published oncogenicity studies on 2,4-D, one in rats (Hanson et al. 1971) and the other in mice (Innes et al. 1969). These studies are inadequate to assess the carcinogenicity of 2,4-D. In study B, a relative risk of 4.9 (90% confidence interval 1.6 - 11.1)* was found in relation to exposure to phenoxy, acid herbicides other than 2,4,5-T (2,4-D, MCPA, mecoprop, dichloroprop).

Relative risks in relation to the three major categories of exposure are shown in Table 40.** Studies A and B indicate a risk of developing soft tissue

*Test-based method of Miettinen (1976); chi-square statistic, no continuity correction.

**In the analyses considering phenoxyacetic acids only and chlorophenols only, persons exposed to the other category of substances were excluded. In study A, the three persons exposed to both chlorophenols and phenoxyacetic acids were included in all comparisons.

TABLE 39. EXPOSURE FREQUENCIES IN TWO CASE-CONTROL STUDIES OF SOFT-TISSUE SARCOMA

Substance(s)	Percent Exposed			
	Study A		Study B	
	Cases (n=52)	Controls (n=206)	Cases (n=110)	Controls (n=219)
Phenoxyacetic acids only	23.1	6.3	12.7	2.3
Chlorophenols only	11.5	2.4	10.0	3.6
Both	1.9	0.5	0	0
Total	36.5	9.2	22.7	5.9

Sources: Study A, Hardell and Sandstrom (1979)
Study B, Eriksson et al. (1979)

TABLE 40. RELATIVE RISKS OF SOFT TISSUE SARCOMA IN RELATION TO EXPOSURE TO PHENOXYACETIC ACIDS AND CHLOROPHENOLS IN TWO CASE-CONTROL STUDIES

	Phenoxyacetic acids only		Chlorophenols only		Phenoxyacetic acids and/or chlorophenols	
	Study A	Study B	Study A	Study B	Study A	Study B
Relative risk[a]	5.3	6.8	6.6	3.3	5.7	4.7
90% Confidence interval	2.7-10.2	3.1-14.9	2.8-15.6	1.6-7.0	3.2-10.2	2.7-8.3

[a]Unmatched odds ratio

[b]Test-based method of Miettinen (1976); chi-square statistic, no continuity correction.

Sources: Study A, Hardell and Sandstrom (1979)
Study B, Eriksson et al. (1979)

sarcomas among workers exposed to phenoxyacetic acids only, chlorophenols only, or phenoxyacetic acids and/or chlorophenols several times higher than among persons not exposed to these chemicals. In each comparison, the point estimate of relative risk is high and unlikely to have resulted by chance alone.

Little is known of the etiology of soft tissue sarcoma, so the consideration of confounding in these studies is largely a hypothetical matter. Age, sex, and place of residence were eliminated as possible confounding factors in the selection of controls.* Because of the high correlation between exposure to the substances of interest and employment in agriculture and forestry, a reasonable hypothesis could be developed that some unknown factor present in these occupations was responsible for the elevated relative risks.

To test this hypothesis, it is possible to calculate the relative risk in relation to phenoxyacetic acid exposure in Study B, restricting the analysis to workers within agriculture and forestry. The result is a relative risk of 6.1 (90% confidence interval 2.4 to 15.4). This finding strongly suggests that a confounding risk factor for soft tissue sarcoma distributed throughout agriculture and forestry work was not responsible for the overall increase in risk found in relation to phenoxyacetic acid exposure.

*Controls were matched individually to cases on the basis of these factors. Unmatched analyses are presented in Table 40 for the sake of simplicity. The matched-method relative risks for exposure to phenoxyacetic acids and/or chlorophenols were 6.2 (90% confidence interval 3.4-11.2) in Study A and 5.1 (90% confidence interval 2.8-9.3) in Study B.

Because exposure histories were obtained by means of q stionnaires and interviews, the major potential source of bias in these st. ies stems from the need to rely upon the personal recollection of cases and controls for exposure histories. The published papers indicate that the researchers paid a great deal of attention to this potential problem and state that they took all reasonable precautions to avoid it during the conduct of the study.

In addition, the relative risk calculated by considering the agriculture and forestry workers who did not report exposure to phenoxyacetic acids or chlorophenols and comparing them to unexposed persons in other occupations was 0.9 (90% confidence interval 0.3 to 2.4) in Study B. This suggests that a great deal of recall bias was not present (Axelson 1980).

Of additional interest are the reports of soft tissue sarcoma deaths in two cohort studies of workers exposed to TCDD. In a study of workers exposed to TCDD during and after a 1949 trichlorophenol process accident in a Monsanto Company facility at Nitro, West Virginia, a death from fibrous histiocytoma was reported (Zack and Suskind 1980). This cause of death was noted by the authors as a rare event. In a study of Dow Chemical Company workers exposed to TCDD in a 1964 "chloracne incident" in a trichlorophenol production area, one of the three cancer deaths was due to fibrosarcoma (Cook et al. 1980). These two deaths from this rare form of cancer support the inference from the Swedish case-control studies that soft-tissue sarcoma may be a hazard of exposure to TCDD. These and others cohort studies should be continued to determine whether additional deaths from soft-tissue sarcoma develop.

The associations reported in the two case-control studies of soft tissue sarcoma are great enough that they are unlikely to have resulted entirely from random variation, bias, or confounding, even though the possibility cannot be completely dismissed that bias or confounding was present. These results are

supported by the occurrence of individual cases of soft-tissue of sarcoma, usually a relatively rare form of cancer, in two cohort studies of workers exposed to TCDD and trichlorophenol. Therefore, the studies provide a strong suggestion that phenoxyacetic acid herbicides, chlorophenols, and/or TCDD are carcinogenic in humans.

MALIGNANT LYMPHOMA

A separate series of clinical observations at the Department of Oncology in Umea, Sweden (Hardell 1979) led the researchers to conduct a case-control study of malignant lymphoma in relation to phenoxyacetic acids, chlorophenols, and other organic compounds (Hardell et al. 1980). Approximately one-third of the cases in this study were patients with Hodgkin's disease; the remainder of the lymphomas were non-Hodgkin's forms. MacMahon (1966) and, more recently, Gutensohn and Cole (1980) have stated that late adult-onset Hodgkin's disease and the other forms of lymphoma are likely to share similar etiologies.

This study employed essentially the same methods and achieved results closely comparable to the soft tissue sarcoma studies: fivefold to sixfold relative risks in relation to phenoxyacetic acids and chlorophenols considered separately or together. In addition, an elevated relative risk was found in connection with exposure to organic solvents such as benzene, trichloroethylene, and styrene. In the published report, the methods and results were incompletely documented, especially the possibility of confounding by exposure to the organic solvents. The researchers indicate that an additional report of this study is in preparation.

Other research has tentatively suggested that lumberjacks may be at increased risk of lymphoma (Edling and Granstam 1980). In addition, the Zack and Suskind study of workers exposed to TCDD found three deaths from cancers of

the lymphatic and hematopoietic system, against only 0.88 death expected (relative risk 3.4, 90% confidence interval 0.9-8.8)*

The lymphoma case-control study (Hardell et al. 1980) is consistent with the two soft-tissue sarcoma studies discussed above. On the other hand, the consistency could also reflect an as yet unidentified methodologic bias in all these studies. It would be useful for a study using the same methods to be conducted on a type of cancer not suspected as a hazard of exposure to phenoxyacetic acids or chlorophenols. If such a study did not show a comparably elevated relative risk, the likelihood that some methodologic source of bias produced the results of the soft-tissue sarcoma and lymphoma studies would be extremely low.

STOMACH CANCER

Studies of two of the oldest cohorts of workers known to have been exposed to phenoxyacetic acid herbicides and/or TCDD report stomach cancer mortality rates higher than expected, but the results in each study are based on small numbers of deaths. In one study (Axelson et al. 1980), 348 Swedish railroad workers with at least 46 days of herbicide exposure between 1955 and 1972 were followed through October 1978. The workers were grouped on the basis of their primary herbicide exposures: those exposed to phenoxyacetic acids (2,4-D and 2,4,5-T) only, to amitrole (aminotriazole) only, and to both types of herbicides.

In addition to the overall results, the authors were able to provide data according to the preferred practice of limiting observation to the time

*Obtained by exact Fisher method (Rothman and Boice 1979).

following a minimum period of cancer induction -- in this case, 10 years from first exposure. The results are shown in Table 41. Expected deaths were derived from Swedish national mortality rates specific for age, sex, and calendar year.

TABLE 41. STOMACH CANCER MORTALITY IN A GROUP OF SWEDISH RAILROAD WORKERS EXPOSED TO HERBICIDES, 10 OR MORE YEARS FROM ONSET OF EXPOSURE

Exposure category	Stomach cancer deaths		Relative risk	90% confidence interval
	Observed	Expected		
Phenoxy acids	2	0.33	6.1	1.1-19.1
Amitrole	0	0.20	---	-------
Amitrole and phenoxies	1	0.18	5.6	0.3-26.4

Source: Axelson et al. (1980)

The estimate of relative risk of stomach cancer for workers with primary exposure to phenoxyacetic acids, but not amitrole, is 6.1. Although this estimate is based on small numbers, the one-tailed Poisson test suggests that it is not likely to have arisen by chance alone (P = 0.044).

The group of all workers with exposure to the phenoxyacetic acids, including those who also had amitrole exposure, had a relative risk of stomach cancer of 5.9 (90% confidence interval 1.6-15.2, three observed stomach cancer deaths, 0.51 expected).

The other study showing increased stomach cancer mortality is the follow-up of 75 workers exposed to TCDD during and after a 1953 runaway reaction at a trichlorophenol manufacturing facility in Ludwigshafen, Federal Republic of

Germany (Thiess and Frentzel-Beyme 1977). In this study, three stomach cancer deaths occurred in contrast to no deaths from stomach cancer in a control group of 75 men, each matched to cohort members by age and date of entry into employment and selected at random from a list of over 10,000 persons working in the same plant. This result indicates an excess of stomach cancer mortality in the exposed workers.

The researchers also derived expected numbers of deaths for this cohort from national and regional mortality rates,* yielding results more readily comparable to those from the Swedish cohort study discussed above. In this analysis, using 1970-1975 rates for the region in which the plant is located and allowing for a 10-year minimum induction period, the researchers found a relative risk of stomach cancer of 7.5 (90% confidence interval 2.0-19.4, 3 observed stomach cancer deaths, 0.40 expected). Although strong temporal trends in the regional stomach cancer mortality rates between 1963 (the start of the follow-up period in this analysis) and 1970 (the beginning of the period covered by the comparison rates) would render the expected death figure somewhat inaccurate, this result confirms the observation of the matched comparison group and are consistent with the Swedish cohort study.

In summary, the evidence that phenoxyacetic acids and/or TCDD might increase the risk of stomach cancer consists of two studies, each of which reports an excess that is based on only three stomach cancer deaths. Further follow-up of these and similar cohorts is warranted, but firm conclusions cannot be made on the basis of the available data.

*An earlier version of the report also included expected deaths calculated from municipal mortality rates, but these were later found to be inaccurate.

OTHER STUDIES

Four additional cohort studies have examined cancer mortality rates in groups of workers exposed to phenoxyacetic acids and/or TCDD. These are a study of Dow Chemical Company 2,4,5-T production workers (Ott et al. 1980), a study of Finnish phenoxyacetic acid herbicide applicators (Riihimaki et al. 1978), and two studies in which trichlorophenol production workers were exposed to TCDD: the previously mentioned Nitro study (Zack and Suskind 1980) and study of Dow Chemical Company employees (Cook et al. 1980).

As noted above, the Nitro study showed a suggestive increase in lymphatic and hematopoietic cancer mortality. In addition, the Nitro study and the study by Cook et al. each included a single death from soft-tissue sarcoma.

The CAG has determined that three of these studies as evidenced by the extremely small numbers of expected cancer deaths in each, have such low statistical power that they cannot be taken as strong evidence of the absence of increased carcinogenic risk in the groups of people studied. In the Nitro study, 9.04 deaths from all malignant neoplasms and only 0.5 from stomach cancer were expected. If the researchers had allowed for a minimum period of cancer induction, these figures would have been even lower. In the study by Ott et al., only 2.6 deaths from all malignant neoplasms were expected with allowance for a 10-year minimum induction perod. The study by Cook et al., with only 1.6 expected deaths from all forms of cancer without allowance for a minimum induction period, had the lowest chance of detecting an effect of all three studies.

Statistically, the study of Finnish herbicide applicators is inconsistent with the results of the Swedish and West German cohort studies. Without regard for induction periods, this study reported 34.5 expected deaths from all malignant neoplasms. The study, therefore, appears powerful enough to detect

relative risks even smaller than those seen in the Swedish and West German studies. A partial explanation for this apparent inconsistency could lie in the fact that the Finnish study set the minimum period of herbicide exposure for membership in the cohort at 10 days (two working weeks) and noted that the "total length of exposure has, in most cases, been a few weeks only." The Swedish study of herbicide applicators set the minimum exposure period at 46 days (>1 spraying season).

There are also certain inconsistencies in the data from the Finnish study which the authors note but find difficult to explain. In particular, no cancer deaths occurred during the latter part of the study period among Forestry Authority workers (one of four groups included in the cohort), even though 9.0 deaths were expected. This finding strongly suggests some deficiency in follow-up or in the source records from which vital status was determined.

In summary, four additional cohort studies of workers exposed to phenoxyacetic acid herbicides and/or TCDD do not report increased risks of stomach cancer. Only one of these, however, was statistically powerful enough to be inconsistent with the two studies that tentatively suggest an increase in stomach cancer risk. The available report of this study of Finnish herbicide applicators contains methodologic questions that require clarification.

SUMMARY

Two Swedish case-control studies report highly significant associations between soft tissue sarcoma and exposure to phenoxyacetic acid herbicides and/or chlorophenols. These studies provide strongly suggestive evidence for the carcinogenicity of 2,4,5-T and/or TCDD. The degree of bias or confounding necessary to produce the highly elevated relative risks in these studies is not likely to have occurred. Weaker evidence exists from epidemiological studies

that exposure to 2,4,5-T and/or TCDD may also increase the risk of malignant lymphoma and stomach cancer in humans. Published studies that have not shown increases cancer mortality among workers exposed to 2,4,5-T and/or TCDD have low statistical power and, therefore, do not provide strongly contradictory evidence.

QUANTITATIVE RISK ASSESSMENT

I. INTRODUCTION

This section assesses the carcinogenic risk posed to humans by 2,4,5-T, silvex, and the contaminant TCDD as a result of the use of commercial 2,4,5-T and silvex in the U.S. The highly suggestive evidence of carcinogenicity both in humans and in animals for 2,4,5-T and the substantial animal evidence for TCDD dictates that an estimate of risk be made for these chemicals. For silvex itself there is no direct evidence of carcinogenicity so that the quantitative estimate of risk will be calculated only for the TCDD contaminant of silvex. Since TCDD may also act as a promoter or cocarcinogen an additional cancer risk exists that is dependent upon exposures to other agents. However, since there is no theoretical basis for quantitatively estimating the risk posed by cancer promoters or cocarcinogens, this potential is not considered in the quantitative estimate of risk.

The individual estimated risks are what we consider to be the maximum plausible primary cancer hazard due to the use of the herbicides 2,4,5-T and silvex for a given exposure. This opinion is based upon the conservative manner in which the data used in the extrapolation are selected, the form of the dose-response model employed, and the conservative assumptions used in estimating the total years of exposure.

It is well recognized that the risk estimates given are only a rough measure of the true potential danger, but even so they should be more useful than no quantified estimate of hazard.

The resulting risk estimates should be viewed as plausible upper bounds while the plausible lower bound, due to the possibility of highly non-linear behavior at low doses, is virtually zero. Since all other mathematical models

presently in use which conform to commonly accepted principles of chemistry and biology would give risk estimates within this range, we feel that their employment would not provide any additional useful information.

This risk assessment is based on two main elements: 1) a mathematical model for extrapolation of animal to human dose-response was developed which can be utilized to estimate risk given an average lifetime exposure to the herbicides, and 2) estimates of the lifetime average exposure to various use patterns of the herbicides were made.

The mathematical model is based on the rationale explained in the "Carcinogen Assessment Group's Method for Determining the Unit Risk Estimate for Air Pollutants," July 31, 1980 (Appendix G). All the experimental animal data for 2,4,5-T and TCDD considered in the employment of the model are fully explained and the results obtained are given in the next section.

The estimated human exposures from the use of these herbicides were supplied to the CAG by the Hazard Evaluation Division (HED) of the Office of Pesticide Programs of EPA and is attached as Appendix F. These estimates were used as given except for the changing of units to mg/kg body wt/day, the appropriate unit for the mathematical model. All of the qualifications, liabilities, assumptions, and reservations about the exposure estimate expressed in the HED document should be kept in mind in evaluating CAG's risk assessment since they naturally apply to all situations where the exposure estimates are utilized.

Also, quantitative estimates of risk were made for only certain uses and routes of exposure of commercial 2,4,5-T and silvex. The CAG's analysis is confined to those situations where HED had sufficient information to generate an exposure estimate.

Risks are estimated below for exposure to workers in forestry, range and brush control, rice-weed control, on rights-of-way, and for exposure to the general population and local populations through the diet by contaminated food.

No estimates ae made of risk due to general environmental exposure.

II. ESTIMATION OF THE DOSE-RESPONSE MODEL

In order to estimate the slope of the dose-response relationship utilizing the linearized multistage model, it is necessary for each study to:

1) Obtain the number of animals that have one or more tumors of all types that are judged to be statistically significantly greater in a test group than in the control group at the 0.05 level as determined by the Fisher Exact 2 x 2 Test, or an exact trend test, and are also judged to be biologically meaningful responses to a carcinogen.

2) Fit the multistage model to these data and test the goodness of fit utilizing the chi-square statistic. If the fit is rejected at the 0.01 level, the data are refitted with the highest dose omitted. The process is continued until an acceptable fit is obtained. For that data set, the largest linear component that is still consistent with the observed data, which may be viewed as a 95% upper bound, is used as the slope estimates upon which the risk estimates are based.

3) These linear components are next translated into human equivalent slope estimates where exposure to humans is in terms of mg/kg body wt/day by multiplying the animal slope estimate by the cube root of the ratio of human to animal weight, where a 70 kg human is assumed.

4) The maximum of these human equivalent slope estimates for all the data sets is then selected to be used as the slope of the linear or more generally the one-hit model from which the risk estimates will be made.

In Tables 42-48, all of the tumor data that were judged to be statistically elevated over control are presented. In Tables 49-58, the data actually used to fit the models, the X^2 value for the goodness of fit test, the fitted

parameters of the multistage model, the upper bound linear component, and the human linear component are all shown for each data set.

In Table 59, the final human slope estimate is given for each data set. The maximum slope factor for all the data sets are 1.82×10^{-2} $(mg/kg/day)^{-1}$ for 2,4,5-T and 4.25×10^{5} $(mg/kg/day)^{-1}$ for TCDD which are used in the risk estimation of all subsequent risk.

The slope for TCDD for 2,4,5-T spray applicators may be converted to be used for exposure given in terms of 2,4,5-T by multiplying the assumed TCDD contamination rate of 2,4,5-T, 4×10^{-8}, by 4.25×10^{5}, the slope for TCDD, giving a value of 1.70×10^{-2} $(mg/kg/day)^{-1}$.

Under these assumptions an estimate of the lifetime probability of cancer for an applicator due to exposure to a lifetime average exposure of x mg/kg/day of commercial 2,4,5-T is

$$P = 1 - e^{-(B_1 + B_2)x}$$

where B_1 = is the maximum converted human slope for TCDD and B_2 = is the maximum human slope for 2,4,5-T alone, or

$$P = 1 - e^{-(0.0170 + 0.0182)x} = 1 - e^{-0.0352x}$$

For applicator exposure to silvex, the risk equation in that case is related only to the TCDD contaminant

$$P = 1 - e^{-B1\ x} = 1 - e^{-0.017x}$$

As discussed in detail in the exposure document, the TCDD contaminant of both 2,4,5-T and silvex is assumed to be present at 40 ppb only for the sprayer

applicator exposure. For dietary exposure due to subsequent contamination of food, except for rice where silvex is used to predict 2,4,5-T levels, measured TCDD levels are used for exposure estimates. This is because the environmental breakdown rates of TCDD and 2,4,5-T are sufficiently different so that the contamination rate of 2,4,5-T would not be predicted from the observed TCDD levels.

III. RISKS FOR APPLICATORS

Risks to workers in forestry, rangeland, rice levies, and rights-of-way spraying with 2,4,5-T and silvex have been estimated. As noted above, TCDD has been assumed to be present as a contaminant in 2,4,5-T and silvex at 40 ppb. For forestry patterns, 2,4,5-T exposure has been measured in workers by Lavy as noted by HED. These measurements have also been assumed for other sprayers of range, rice levies, and rights-of-way. These exposures and associated risks are shown in Tables 60 and 61.

Worker exposure to silvex has not been measured but silvex has been assumed to be absorbed into the body in the same manner as 2,4,5-T. Thus, exposure is also estimated by using the Lavy 2,4,5-T data discussed by HED. It is assumed that the applicators are exposed to the same number of hours each year that apply 2,4,5-T as those that apply silvex and the number of workers applying silvex compaired to 2,4,5-T is the same as the usage ratios presented by HED (pg. 13 Appendix F) and shown below. As a result risk to applicators applying silvex would be equivalent to the risks based upon TCDD contaminant which is shown in Tables 60 and 61.

Uses	2,4,5-T:Silvex ratio
Rangeland/pasture	10:1
Forestry	100:1
Rice	1000:1
Rights-of-way	10:1

FORESTRY

For forestry sprayers, risks based on measured exposure are shown in Table 60. Lavy gives the exposure as total dose based on the actual clearance of 2,4,5-T from 21 workers. Based on total hours exposed per year and total worker population exposed and an assumed 40 year working life, a total lifetime exposure was estimated and lifetime cancer risks have been extrapolated. The upper limits on these lifetime risks range from 10^{-4} to 10^{-3} with the highest risk associated with the aerial mixer-loaders, 2.7×10^{-3}. The small number of workers exposed, however, results in a very small number of cases per year, even under the assumption of a 40-year working lifetime. Furthermore, the above analysis does not assume protective clothing.

RANGE AND BRUSH CONTROL

Based on estimated exposure for unprotected range sprayers, Table 61 shows upper limits on lifetime risks of 10^{-6} to 10^{-4}, with the highest risk of 1.7×10^{-4} to the mixer/loaders. With only 200 of these estimated, however, the estimated annual case rate is essentially 0. The risk to each of the 20,000 backpack sprayers is estimated to be 3.5×10^{-6}.

RICE-WEED CONTROL

Based on the measured exposure from the forestry workers, adjusted for application rates of the active ingredient 2,4,5-T, the estimated lifetime risks are presented in Table 61. These estimated risks for unprotected workers are

in the 10^{-8} to 10^{-5} range, with the highest risk of 7.8×10^{-5} to the mixer/loaders. These risks are about one-half to one order of magnitude less than those for the range sprayers due to the less time exposed.

RIGHTS-OF-WAY BRUSH AND WEED CONTROL

Based on the measured exposure from the forestry worker, adjusted for application rates of the active ingredient 2,4,5-T, the estimated lifetime cancer risks for these unprotected workers are presented in Table 61. These risks are about one-half an order of magnitude greater than the risks for the forestry workers. This is mainly because of the higher concentration of 2,4,5-T used in the application. The upper limit risks for these unprotected workers is in the 10^{-4} to 10^{-3} range.

IV. RISKS DUE TO DIETARY EXPOSURE

Human exposure to TCDD contamination of foods by 2,4,5-T and silvex is presented. There are four areas where HED has reported TCDD levels: beef fat, cows' milk, deer and elk fat. HED has also presented an analysis of 2,4,5-T contamination of milk and rice. The CAG has used these estimates.

BEEF AND MILK

Two monitoring studies for dioxin in beef fat have been discussed by HED, with emphasis on the Phase One Beef Study of the EPA Dioxin Implementation Plan, 1975. In this study 67 samples of beef were analyzed. Based on a correlation of those samples analyzed by high resolution mass spectomotry and the application rate of the herbicide, HED projects a residue of 4.2 ppt TCDD in beef fat for cattle and cows grazing on land treated that year with 2,4,5-T at a rate of 1 lb/acre active ingredient. If the land were to be treated with

2,4,5-T at a higher rate, up to the legal limit of 4 lb/acre, both the residues and associated risks would be correspondingly higher.

Based on the 4.2 ppt TCCD contamination level in beef fat and a beef consumption of approximately 100 lb/person/year, HED estimates that TCDD dietary intake from beef for the general population is approximately 0.4 pg/day. For the local population consuming only contaminated beef, dietary intake could be as high as 31 pg TCDD/person/day assuming a 5-year treatment cycle.

Likewise, for milk contamination, assumption of 4.2 ppt TCDD in fat of grazing cows would project to as much as 74 pg TCDD/day dietary intake for local populations or for those consuming only contaminated dairy products. Measurements of silvex in milk assumed similar for 2,4,5-T, yield exposure estimates of 7.1 ng/kg/day 2,4,5-T for the local population.

Based on the above exposure estimates Table 62 shows that the upper limit risk estimates for beef contamination at the above estimated exposures are 1.9×10^{-4} for the local population and 2.4×10^{-6} for the general population. For the general population this gives an upper limit number of cases of 7.5/year. For milk and dairy products the upper limit risk estimate for estimated exposures is 4.7×10^{-4} for the average consumer of only contaminated products.

DEER AND ELK

HED has estimated the dietary intake from TCDD contaminated deer and elk meat to be between 0.14-9.3 pg/kg/meal for deer and 0.05-20.5 pg/kg/meal for elk. All consumption is assumed to be by the local population of hunters and their families. The maximum projected risks based on 12 meals per year for life are 1.3×10^{-4} for deer and 2.9×10^{-5} for elk. These are presented in Table 63. More or less consumption would lead to corresponding increases or decreases in risk.

RICE

Based on a possible residue of 2,4,5-T on rice of 12 ppb, 10.9 percent crop treated annually and a food factor of 0.55 percent, HED estimates the average intake of 2,4,5-T from contaminated rice as 0.011 ug/day for the general population. On a dose/body weight basis this becomes 0.154 ng/kg/day for a 70 kg person consuming 1.5 kg food/day. HED also estimates that a high consumer of rice could ingest ten times as much rice and correspondingly, ten times as much 2,4,5-T. These estimates and results are presented in Table 64. the risks are from 2.8×10^{-9} to 2.8×10^{-8} in the general population to 2.5×10^{-8} to 2.5×10^{-7} in the high consumer group.

IV. SUMMARY

A quantitative assessment has been calculated for the carcinogenic risk posed to humans by the use of the herbicides 2,4,5-T and silvex. While there is no evidence for carcinogenicity of silvex, the evidence for 2,4,5-T is highly suggestive, and that for the contaminant TCDD is substantial. Furthermore, TCDD is highly carcinogenic to animals.

The assessment of risk from TCDD exposure covers only the herbicide applicators and dietary exposure to beef, milk, deer, and elk. For unprotected workers, the upper limits of lifetime risk of induced cancers are in many cases as high as or in the 10^{-3} range. For the general population exposed to contaminated beef, the upper limit of risk for the estimated exposure is 2.4×10^{-6}. For local populations consuming only beef which is contaminated with TCDD, the risk is much greater, as high as 1.9×10^{-4}. For local populations consuming only milk and other dairy products which are contaminated with TCDD, the risk is 4.7×10^{-4}.

For contaminated deer and elk meat, risks to the local population are no greater than 10^{-4} for 12 meals a year.

The upper limit of dietary risk associated with estimated exposure to 2,4,5-T in contaminated rice and milk were in the 10^{-7} range for a high consumer eating only contaminated rice or an average consumer drinking only contaminated milk.

TABLE 42. DOW (DR. KOCIBA) TCDD ORAL RAT STUDY (1978) WITH DR. R. SQUIRE'S REVIEW
Male Sprague-Dawley Rats - Spartan Substrain (2 yrs.)[a]
MALES

Tissue and Diagnosis	Dose Levels (ug/kg/day)			
	0 (control)	0.001	0.01	0.1
Dow (Kociba) Analysis				
1. Tongue Stratified squamous cell carcinoma	0/76	1/49	1/49	3/42 ($P = 0.043$)
2. Nasal Turbinates/Hard Palate Squamous cell carcinoma	0/51	1/34	0/27	4/30 ($P = 0.016$)
Total	0/76	2/49	1/49	7/42 ($P = 5.12 \times 10^{-4}$)
R. Squires Review				
1. Tongue Squamous cell carcinoma	0/77	1/44	1/49	3/44 ($P = 4.60 \times 10^{-2}$)
2. Nasal Turbinates/Hard Palate Squamous cell carcinoma	0/55	1/34	0/26	6/30 ($P = 1.36 \times 10^{-3}$)
Total - 1 or 2 above (each rat had at least one tumor above)	0/77	2/44	1/49	9/44 ($P = 6.28 \times 10^{-5}$)

[a]Average body weight of male rat = 600 grams

TABLE 43. DOW (DR. KOCIBA) TCDD ORAL RAT STUDY (1978) WITH DR. R. SQUIRE'S REVIEW
Female Sprague-Dawley Rats - Spartan Substrain (2 yrs.)[a]

FEMALES

Tissues and Diagnoses	Dose Levels (ug/kg/day)			
	0 (control)	0.001	0.01	0.1
Dow (Kociba) Analysis				
1. Lung Keratinizing squamous cell carcinoma	0/86	0/50	0/49	7/49 ($P = 6.21 \times 10^{-4}$)
2. Nasal Turbinates/Hard Palate Stratified squamous cell. carcinoma (Revised diagnoses 2/19/79)	1/54	0/30	1/27	5/24 ($P = 9.46 \times 10^{-3}$)
3. Liver Hepatocellular hyperplastic nodules/hepatocellular carcinoma	9/86	3/50	18/50 (2 had both) ($P = 4.37 \times 10^{-4}$)	34/48 ($P = 9.53 \times 10^{-13}$)
Total 1, 2, or 3 above (each rat had at least one tumor above)	9/86	3/50	18/50 ($P = 4.37 \times 10^{-4}$)	34/49 ($P = 2.13 \times 10^{-12}$)

[a]Average body weight of female rat = 450 grams.

(continued on following page)

TABLE 43. (continued)

R. Squire's Review				
1. Lung Squamous cell carcinoma	0/86	0/50	0/49	8/47 ($P = 1.61 \times 10^{-4}$)
2. Nasal Turbinate/Hard Palate Squamous cell carcinoma	0/54	0/30	1/27	5/22 ($P = 1.43 \times 10^{-3}$)
3. Liver Neoplastic nodules/ hepatocellular carcinoma	16/86	8/50	27/50 ($P = 2.42 \times 10^{-5}$)	33/47 ($P = 4.92 \times 10^{-9}$)
Total Combined (1, 2, or 3 above) (each animal had at least one tumor above)	16/86	8/50	27/50 ($P = 2.42 \times 10^{-5}$)	34/47 ($P = 1.20 \times 10^{-9}$)

[a]Average body weight of female rat = 450 grams

TABLE 44. NCI TCDD (GAVAGE) BIOASSAY (#80-1765)
Osborne-Mendel Rats (2 yrs.) W = 700 g

MALES[a]

Tissues and Diagnoses	Dose Levels (ug/kg/wk) vehicle control 0	low 0.01	medium 0.05	high 0.5
1. Adrenal Cortical adenoma[b]	6/72	9/50 (P = 0.093) N.S.[c]	12/49 (P = 0.015)	9/49
2. Thyroid Follicular cell adenoma carcinoma	1/69	5/48 (P = 0.042)	8/50 (P = 0.004)	11/50 ($P = 2.84 \times 10^{-4}$)

[a]Subcutaneous combined fibroma or fibrosarcoma - not significant.

[b]The biological significance of this tumor in old age rats is questionable, since it is commonly observed in control rats and is associated with the aging process.

[c]N.S. = Not significant.

TABLE 45. NCI TCDD (GAVAGE) BIOASSAY (#80-1765)
Osborne-Mendel Rats (2 yrs.) W = 450 g

FEMALES

Tissues and Diagnoses	Dose Levels (ug/kg/wk) vehicle control 0	low 0.01	medium 0.05	high 0.5
1. Liver				
Neoplastic nodule or hepatocellular carcinoma	5/75	1/49	3/50	14/49 (P = 0.001)
2. Adrenal[a]				
Cortical adenoma, or carcinoma	11/73	9/49	5/49	14/46 (P = 0.038)

[a]The biological significance of this tumor in old age rats is questionable, since they are commonly observed in control rats and associated with aging process.

TABLE 46. NCI TCDD (GAVAGE) BIOASSAY (#80-1765)
B6C3F1 MICE (2 yrs.) W = 48 g

MALES

Tissue and Diagnosis	Dose Levels (ug/kg/wk)			
	vehicle control 0	low 0.01	medium 0.05	high 0.5
Liver				
Hepatocellular adenoma or carcinoma	15/73	12/49	13/49	27/50 ($P = 1.31 \times 10^{-4}$)

TABLE 47. NCI TCDD (GAVAGE) BIOASSAY (#80-1765)
B6C3F1 MICE (2 yrs.)

FEMALES[a]

Tissues and Diagnoses	Dose Levels (ug/kg/wk) vehicle control 0	low 0.04	medium 0.2	high 2.0
1. Subcutaneous tissue Fibrosarcoma	1/74	1/50	1/48	5/47 ($P = 0.032$)
2. Hematopoietic system Lymphoma or leukemia	18/74	12/50	13/48	20/47 ($P = 0.028$)
3. Liver Hepatocellular adenoma or carcinoma	3/73	6/50	6/48	11/47 ($P = 1.84 \times 10^{-3}$)
4. Thyroid Follicular cell adenoma	0/69	3/50	1/47	5/46 ($P = 8.93 \times 10^{-3}$)
Total 1, 2, 3 or 4 above (each mouse had at least one tumor above)	22/74	20/50	19/48	31/47 ($P = 8.94 \times 10^{-5}$)

[a]Average body weight of female mouse = 40 grams

TABLE 48. DOW (DR. KOCIBA) 2,4,5-T ORAL RAT STUDY (1978)WITH DR. SQUIRE'S REVIEW
Sprague-Dawley Rats - Spartan Substrain

MALES[a]

Tissue and Diagnosis	Dose Levels (mg/kg/day) 0 (control)	3	10	30
Dow (Dr. Kociba) Analysis				
Tongue				
Stratified squamous cell carcinoma	1/83	1/50	0/46	4/49 (P = 0.063)
Dr. R. Squire's Review				
Tongue				
Squamous cell carcinoma	1/83	1/50[b]	0/46[b]	5/48 (P = 0.025)

[a]Average weight of male rat = 600 grams

[b]Dr. Squire examined all slides from the middle and low dose described by Dow (original report) as exhibiting any lesions, but did not review tongue slides that Dow described as having no lesions. The incidence numbers for low and medium dose levels in this table represent this combined review incidence (i.e., Dow's tongue diagnoses confirmed by Dr. Squire).

TABLE 49. CURVE FIT OF THE MULTISTAGE MODEL PARAMETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOGIST. LINEAR PARAMETER q_1, MAXIMIZED TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....Kociba - Dow
Sex-species.....Male rat
Weight (w_a).....600 gm
Tumor sites (one or more)....Tongue - squamous cell carcinomas

Pathologist - Kociba

Exposure level (mg/kg/day)	0	1×10^{-6}	1×10^{-5}	1×10^{-4}
+r/n[a]	0/76	1/49	1/49	3/42

+r = number of animals with one or more of the tumors
n = total number of animals examined
[a]Because of an error discovered just before press time, the above data cover only the squamous cell carcinomas of the tongue and omit from the numerator those animals which developed only nasal turbinate and hard palate tumors. Preliminary calculations have satisfied us, however, that these data would not have provided us with the maximum slope factor used in the TCDD quantitative risk assessment.

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.84×10^{-2}	0.70×10^{3}	0	0	1.68×10^{3}	1.41 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P > 0.01$) = 1.68×10^{3}

$q_1^* = q_1^* (70/w_a)^{1/3} = 8.21 \times 10^{3}$, the upper 95% limit one-hit slope factor associated with human dose response.

ABLE 50. CURVE FIT OF THE MULTISTAGE MODEL PAR ERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOG
LINEAR PARAMETER q_1, MAXIMIZ.. O GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....Dow
Sex-species.....Male rat
Weight (w_a).....600 gm
Tumor sites (one or more)....Nasal turbinates/hard palate - squamous cell carcinoma
Tongue - squamous cell carcinomas

Pathologist - Squire

Exposure Level (mg/kg/day)	0	1×10^{-6}	1×10^{-5}	1×10^{-4}
+r/n	0/77	2/44	1/49	9/44

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit χ^2
When all dose groups are used	0.015	1.05×10^3	0	109.40×10^9	3.53×10^3	3.90 (d.f.=1)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P > 0.01$) = 3.53×10^3

$q_h^* = q_1^* (70/w_a)^{1/3} = 1.73 \times 10^4$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 51. CURVE FIT OF THE MULTISTAGE MODEL PARAMETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOGIST. LINEAR PARAMETER q_1, MAXIMIZED TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....Dow
Sex-species.....Female rat
Weight (w_a).....450 gm
Tumor sites (one or more)....Liver, lung, hard palate, or nasal tubinates

Pathologist - Kociba

Exposure level (mg/kg/day)	0	1×10^{-6}	1×10^{-5}	1×10^{-4}
+r/n	9/86	3/50	18/50	34/49

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.12	1.23×10^4	0	0		8.63 (d.f.=2)
When the highest dose group is not used	0.09	0	3.5×10^9		4.69×10^4	0.92 (d.f.=1)
When the two highest dose groups are not used	Above fit is satisfactory					

q_1^* the maximum linear component from the model with adequate goodness of fit ($P > 0.01$) = 4.69×10^4

$q_h^* = q_1^* (70/w_a)^{1/3} = 2.52 \times 10^5$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 52. CURVE FIT OF THE MULTISTAGE MODEL PA :TERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLC I.
LINEAR PARAMETER q_1, MAXIMI TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....Kociba - Dow
Sex-species.....Female rat
Weight (w_a).....450 gm
Tumor sites (one or more)....Liver, lung, hard palate, or nasal tubinates

Pathologist - Squire

Exposure level (mg/kg/day(	0	1 x 10^{-6}	1 x 10^{-5}	1 x 10^{-4}
+r/n	16/86	8/50	27/50	34/47

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.26	1.25 x 10^4	0	0		14.47 (d.f.=2)
When the highest dose group is not used	0.19	0	5.83 x 10^9		7.90 x 10^4	0.209 (d.f.=1)
When the two highest dose groups are not used			Above fit is satisfactory			

q_1^* the maximum linear component from the model with adequate goodness of fit (P > 0.01) = 7.90 x 10^4

$q_1^* = q_1^* (70/w_a)^{1/3}$ = 4.25 x 10^5, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 53. CURVE FIT OF THE MULTISTAGE MODEL PARAMETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOGIST. LINEAR PARAMETER q_1, MAXIMIZED TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....NCI
Sex-species.....Male rat
Weight (w_a).....700 gm
Tumor sites (one or more)....Thyroid - adenoma or carcinoma

Pathologist - NCI Reviewed

Exposure level (mg/kg/day)	1.43×10^{-6}	7.14×10^{-6}	7.14×10^{-5}
+r/n	1/69	5/48	8/50	11/50

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameter	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	7.31×10^{-2}	2.85×10^{3}	0	0	5.24×10^{3}	7.13 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P > 0.01$) = 5.24×10^{3}

$q_1^* = q_1^* (70/w_a)^{1/3} = 2.43 \times 10^{4}$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 54. CURVE FIT OF THE MULTISTAGE MODEL P ETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOL T. LINEAR PARAMETER q_1, MAXIM. TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....NCI
Sex-species.....Female rat
Weight (w_a).....450 gm
Tumor sites (one or more)....Liver tumor

Pathologist - NCI Reviewed

Exposure level (mg/kg/day)	0	1.43×10^{-6}	7.14×10^{-6}	7.14×10^{-5}
+r/n	5/75	1/49	3/50	14/49

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multidtage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.05	0	5.65×10^7	0	6.09×10^3	1.44 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P < 0.01$) = 6.09×10^3

$q_h^* = q_1^* (70/w_a)^{1/3} = 3.28 \times 10^4$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 55. CURVE FIT OF THE MULTISTAGE MODEL PARAMETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOGIST. LINEAR PARAMETER q_1, MAXIMIZED TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....NCI
Sex-species.....Male mice
Weight (w_a).....48 gm
Tumor sites (one or more)....Liver

Pathologist - NCI Review

Exposure level (mg/kg/day)	0	1.43×10^{-6}	7.14×10^{-6}	7.14×10^{-5}
+r/n	15/73	12/49	13/49	27/50

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.25	7.51×10^3	0	0	1.17×10^4	0.16 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P < 0.01$) = 1.17×10^4

$q_h^* = q_1^* (70/w_a)^{1/3} = 1.33 \times 10^5$, the upper 95% limit one-hit slope factor associated with human dose response.

ABLE 56. CURVE FIT OF THE MULTISTAGE MODEL PAI TERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLO T. LINEAR PARAMETER q_1, MAXIMIZ. TO GIVE UPPER 95% LIMIT q_1^*

Compound.....TCDD
Study.....NCI
Sex-species.....Female mice
Weight (w_a).....40 gm
Tumor sites (one or more)....Subcutaneous tissue-fibrosarcoma, hematopoietic system lymphoma, or leukemia; Liver-hepatocellular adenoma or carcinoma; Thyroid-follicular cell adenoma

Pathologist - NCI Reviewed

Exposure level (mg/kg/day)	0	5.71×10^{-6}	2.86×10^{-5}	2.86×10^{-4}
+r/n	22/74	20/50	19/48	31/47

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit χ^2
When all dose groups are used	0.41	2.38×10^3	0	0	3.78×10^3	1.20 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodnes of fit ($P < 0.01$) = 3.78×10^3

$q_h^* = q_1^* (70/w_a)^{1/3} = 4.56 \times 10^4$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 57. CURVE FIT OF THE MULTISTAGE MODEL PARAMETERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLOGIST. LINEAR PARAMETER q_1, MAXIMIZED TO GIVE UPPER 95% LIMIT q_1^*

Compound.....2,4,5-T
Study.....Dow
Sex-species.....Male rat
Weight (w_a).....600 gm
Tumor sites (one or more)....Tongue

Pathologist - Kociba

Exposure level (mg/kg/day)	0	3	10	30
+r/n	1/83	1/50	0/46	4/49

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.01	0	0	2.63×10^{-6}	3.38×10^{-3}	1.04 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P < 0.01$) = 3.38×10^{-3}

$q_1^* = q_1^* (70/w_a)^{1/3} = 1.65 \times 10^{-2}$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 58. CURVE FIT OF THE MULTISTAGE MODEL PAI TERS TO EXPERIMENTAL DATA BY STUDY AND PATHOLO T. LINEAR PARAMETER q_1, MAXIMI. TO GIVE UPPER 95% LIMIT q_1^*

Compound.....2,4,5-T
Study.....Dow
Sex-species.....Male rats
Weight (w_a).....600 gm
Tumor sites (one or more)....Tongue

Pathologist - Squire

Exposure level (mg/kg/day)	0	3	10	30
+r/n	1/83	1/50	0/46	5/48

+r = number of animals with one or more of the tumors
n = total number of animals examined

Estimated multistage parameters	q_0	q_1	q_2	q_3	q_1^*	Goodness of fit X^2
When all dose groups are used	0.01	0	0	3.51×10^{-6}	3.72×10^{-3}	0.94 (d.f.=2)
When the highest dose group is not used	Above fit is satisfactory					
When the two highest dose groups are not used						

q_1^* the maximum linear component from the model with adequate goodness of fit ($P < 0.01$) = 3.72×10^{-3}

$q_1^* = q_1^* (70/w_a)^{1/3} = 1.82 \times 10^{-2}$, the upper 95% limit one-hit slope factor associated with human dose response.

TABLE 59. HUMAN SLOPE ESTIMATES

Compound	Species	Study	Sex	Pathologist	Human Slope Estimate q_h^*
TCDD	Rat	Dow	Male	Kociba	8.21×10^3
				Squire	1.73×10^4
			Female	Kociba	2.52×10^5
				Squire	$4.25 \times 10^{5*}$
		NCI	Male	NCI - Reviewed	2.43×10^4
			Female	NCI - Reviewed	3.28×10^4
	Mice	NCI	Male	NCI - Reviewed	1.33×10^5
			Female	NCI - Reviewed	4.56×10^4
2,4,5-T	Rat	Dow	Male	Kociba	1.65×10^{-2}
				Squire	$1.82 \times 10^{-2*}$

*Values used in risk analysis

2,4,5-T MEASURED EX 'IRE[a] CALCULATED ON AN HOURLY BASIS

Use pattern	Exposed group (number for 2,4,5-T[b])	Dose average mg/kg/hr[b] 2,4,5-T (hrs/yr)	mg/kg/day Lifetime 2,4,5-T	Risk[c] Lifetime 2,4,5-T (pure)	Risk[d] Lifetime based on TCDD contaminant	Total Lifetime risk commerical (2,4,5-T)	Average cases/yr[e] Total 2,4,5-T plus silvex
Forestry							
1. Aerial	Pilots (73)	0.015(200)	4.6×10^{-3}	8.4×10^{5}	7.8×10^{-5}	1.6×10^{-4}	$<10^{-3}$
	Mixer/Loaders (73-145)	0.062(800)	7.6×10^{-2}	1.4×10^{-3}	1.3×10^{-3}	2.7×10^{-3}	0.06
	Supervisors (--)	0.004(800)	4.9×10^{-3}	9.0×10^{-5}	8.4×10^{-5}	1.7×10^{-4}	---
	Flaggers (--)	0.003(800)	3.7×10^{-3}	6.7×10^{-5}	6.3×10^{-5}	1.3×10^{-4}	---
2. Ground broadcast							
a. Tractor mistblower	Mixer/Loaders (180)	0.020(480)	1.5×10^{-2}	2.7×10^{-4}	2.5×10^{-4}	5.2×10^{-4}	0.001
	Driver (90)	0.013(240)	4.8×10^{-3}	8.7×10^{-5}	8.2×10^{-5}	1.7×10^{-4}	$<10^{-3}$
	Supervisor (--)	0.006(480)	4.4×10^{-3}	8.1×10^{-5}	7.5×10^{-5}	1.6×10^{-4}	---
b. Backpack sprayer	Applicator (300)	0.021(800)	2.6×10^{-2}	4.7×10^{-4}	4.4×10^{-4}	9.1×10^{-4}	0.004
	Mixer-supervisor	0.003(800)	3.7×10^{-3}	6.7×10^{-5}	6.3×10^{-5}	1.3×10^{-4}	---

[a]Compared to skin absorption, potential exposure through the lungs was considered negligibly small by Lavy's measurements.

[b]Figures from HED (Appendix F). Numbers exposed for silvex given in text.

$$1 \text{ mg/kg/year for 40 years} = 40 \text{ year} \times \frac{1 \text{ life}}{71.3 \text{ years}} \times \frac{1 \text{ year}}{365 \text{ days}} = 1.54 \times 10^{-3} \text{ mg/kg/day lifetime.}$$

[c]2,4,5-T. Slope = 1.82×10^{-2} $(\text{mg/kg/day})^{-1}$, from Table 59.

[d]TCDD. Slope = 4.25×10^{5} $(\text{mg/kg/day})^{-1}$, from Table 59. This risk is for the TCDD contaminant of both 2,4,5-T and silvex.

[e]Total expected cases 2,4,5-T plus silvex divided by 71.3.

TABLE 61. LIFETIME PROBABILITY OF INDUCED CANCER FOR 2,4,5-T AND SILVEX APPLICATORS BASED ON FORESTRY SPRAYER 2,4,5-T MEASURED EXPOSURE AND ON ASSUMED WORKER EXPOSURE FOR RANGELAND, RICE LEVY AND RIGHTS-OF-WAY SPRAYING[a]

Use pattern	Exposed group (number for 2,4,5-T)	Dose average mg/kg/hr 2,4,5-T (hrs/yr)	mg/kg/day Lifetime 2,4,5-T	Risk Lifetime 2,4,5-T (pure)	Risk Lifetime based on TCDD Contaminant	Total Lifetime risk commerical (2,4,5-T)	Average cases/yr Total 2,4,5-T plus Silvex
Range - brush and weed control							
1. Aerial	Pilots (130)	0.008(75)	9.2×10^{-4}	1.7×10^{-5}	1.6×10^{-5}	3.3×10^{-5}	$2 10^{-4}$
	Mixer/loaders (130)	0.031(100)	4.8×10^{-3}	8.7×10^{-5}	8.1×10^{-5}	1.7×10^{-4}	$<10^{-3}$
	Flaggers (800)	0.002(25)	7.7×10^{-5}	1.4×10^{-6}	1.3×10^{-6}	2.7×10^{-6}	$<10^{-4}$
2. Ground	Backpack-spot applicators (20,000)	0.008(80)	9.8×10^{-4}	1.8×10^{-5}	1.7×10^{-5}	3.5×10^{-5}	.010
Rice - weed control (aerial)							
	Pilots (310)	0.008(12)	1.5×10^{-4}	2.7×10^{-6}	2.5×10^{-6}	5.2×10^{-6}	$<10^{-4}$
	Mixer/loaders (310)	0.030(48)	2.2×10^{-3}	4.0×10^{-5}	3.8×10^{-5}	7.8×10^{-5}	$<10^{-3}$
	Flaggers (farm labor) (6500-9500)	0.0021(0.6)	1.9×10^{-6}	3.5×10^{-8}	3.3×10^{-8}	6.8×10^{-8}	$<10^{-5}$

[a]See notes on previous table.

(continued on following pa

TABLE 61. (continued)

Use pattern	Exposed group (number for 2,4,5-T)	Dose average mg/kg/hr 2,4,5-T (hrs/yr)	mg/kg/day Lifetime 2,4,5-T	Risk Lifetime 2,4,5-T (pure)	Risk Lifetime based on TCDD contaminant	Total Lifetime risk commerical 2,4,5-T	Average cases/yr Total 2,4,5-T plus silvex
Rights-of-way							
1. Aerial	Pilots (25)	0.060(400)	3.7×10^{-2}	6.7×10^{-4}	6.3×10^{-4}	1.3×10^{-3}	$<10^{-3}$
	Mixer/loaders (25-50)	0.240(400)	1.5×10^{-1}	2.7×10^{-3}	2.5×10^{-3}	4.8×10^{-3}	0.004
2. Ground							
a. Selective Basal	Applicators (1380)	0.084(1,000)	1.3×10^{-1}	2.3×10^{-3}	2.2×10^{-3}	4.5×10^{-3}	0.091
b. Cut Stump	Applicators (60)	0.053(500)	4.1×10^{-2}	7.4×10^{-4}	6.9×10^{-4}	1.4×10^{-3}	0.001
c. Mixed	Handgun applicators (270)	0.079(660)	8.0×10^{-2}	1.5×10^{-3}	1.4×10^{-3}	2.9×10^{-3}	0.005
Brush	Truck/Boom applicators (180)	0.005(660)	5.1×10^{-3}	9.2×10^{-5}	8.6×10^{-5}	1.8×10^{-4}	$<10^{-3}$
d. Railroad	Crew (of four) (110)	0.066(260)	2.6×10^{-2}	4.8×10^{-4}	4.5×10^{-4}	9.3×10^{-4}	0.002
e. Electric Power	Applicators (400)	0.080(660)	8.1×10^{-2}	1.5×10^{-3}	1.4×10^{-3}	2.9×10^{-3}	0.017

[a] See notes on previous tables.

TABLE 62. ESTIMATED DIETARY INTAKE IN PG/KG/DAY OF TCDD* AND 2,4,5-T FROM CONTAMINATION OF BEEF AND MILK OF CATTLE AND COWS GRAZED ON 2,4,5-T TREATED RANGE OR PASTURE LAND. ALSO ESTIMATED LIFETIME CANCER RISK FROM CONTINUOUS EXPOSURE, BY LOCAL AND GENERAL POPULATION

Estimated	Beef Fat	Milk and Diary Products
Local Population*		
TCDD pg/kg/day	0.44	1.05
2,4,5-T pg/kg/day	-------	$<7.14 \times 10^{3}$
Estimated Risk	1.9×10^{-4}	4.7×10^{-4}
Exposed Population	7,200-12,300	-------
Average Cases/Year	0.02 - .03	-------
General Population		
TCDD pg/kg/day	5.7×10^{-3}	-------
2,4,5-T pg/kg/day	-------	-------
Estimated Risk	2.4×10^{-6}	-------
Exposed Population	220,000,000	-------
Average Cases/Year	7.5	-------

*Exposure estimates from HED divided by 70 kg for proper unit conversion.

TABLE 63. ESTIMATED INTAKE OF TCDD FROM CONTAMINATION OF DEER AND ELK MEAT BY ANIMALS FORAGING ON 2,4,5-T TREATED LAND ALSO, ESTIMATED LIFETIME CANCER RISKS

	Deer	Elk
Dietary intake pg/kg bw/day for one meal	0.14 - 9.3	0.05 - 20.5
Assumed meals/year*	12	12
Equivalent daily dose pg/kg/bw/day	0.0046 - 0.3058	0.0016 - 0.6740
Estimated risk	2.0×10^{-6}-1.3×10^{-4}	6.8×10^{-7}-2.9×10^{-5}

*For higher or lower consumption, the risk will vary proportionately.

TABLE 64. ESTIMATED INTAKE OF 2,4,5-T FROM CONTAMINATION OF RICE BY LOCAL AND GENERAL POPULATION BY AVERAGE AND HIGH COMSUMER

Rice	Local population		General population	
Amount	Average Consumer	High Consumer	Average Consumer	High Consumer
2,4,5-T ng/kg/day	1.40	14	0.154	1.5
Estimated risk	2.5×10^{-8}	2.5×10^{-7}	2.8×10^{-9}	2.8×10^{-8}
Exposed population	---		220,000,000	
Average cases/year	---		0.009	

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APPENDIX A

TABLE III-7. CUMULATIVE MORTALITY OF MALE RATS
(KOCIBA ET AL. 1977)

Time (end of 30-day period) N=	Controls (86)	ug/kg/day TCDD 0.1 (50)	0.01 (50)	0.001 (50)
1-7	0.0	0.0	0.0	2.0
8	0.0	2.0	0.0	2.0
9	0.0	4.0	0.0	2.0
10	0.0	4.0	0.0	2.0
11	2.3	4.0	0.0	2.0
12	5.8	8.0	0.0	2.0
13	7.0	12.0	0.0	2.0
14	10.5	18.0	4.0	4.0
15	12.8	18.0	14.0	14.0
16	16.3	20.0	22.0	14.0
17	18.6	28.0	28.0	24.0
18	24.4	34.0	34.0	44.0*
19	31.4	44.0	46.0	50.0
20	41.9	46.0	54.0	56.0
21	48.8	62.0	68.0	60.0
22	58.1	74.0*	76.0*	68.0
23	69.8	78.0	84.0	74.0
24	77.9	84.0	88.0	76.0
25	82.6	90.0	92.0	78.0

*Interval of greatest difference, D, in cumulative mortality curves of controls and treatment group. None of the differences were statistically significant (Kolmogorov-Smirnov test, $P > 0.05$).

TABLE III-8. CUMULATIVE MORTALITY OF FEMALE RATS
(KOCIBA ET AL. 1977)

Time (end of 30-day period) N=	Controls (86)	ug/kg/day TCDD 0.1 (50)	0.01 (50)	0.001 (50)
0-5	0.0	0.0	0.0	0.0
6-8	1.2	0.0	0.0	0.0
9	1.2	2.0	0.0	0.0
10	1.2	4.0	2.0	0.0
11	1.2	8.0	2.0	0.0
12	1.2	16.0	4.0	4.0
13	3.5	20.0	4.0	4.0
14	3.5	26.0	8.0	6.0
15	7.0	28.0	12.0	10.0
16	12.8	32.0	18.0	12.0
17	15.1	38.0	18.0	18.0
18	18.6	44.0	20.0	22.0
19	25.6	56.0*	30.0	34.0*
20	34.9	60.0	36.0	36.0
21	40.7	66.0	46.0*	44.0
22	58.1	82.0	60.0	52.0
23	64.0	86.0	66.0	58.0
24	70.9	88.0	72.0	66.0
25	70.9	92.0	72.0	68.0

*Interval of greatest difference, D, in cumulative mortality curves of controls and treatment group. The mortality curve for the rats fed 0.1 ug/kg/day differed significantly from that for controls ($D = 30.4$, $P < 0.01$, Kolmogorov-Smirnov test). The other two groups did not differ significantly from controls ($P > 0.05$).

TABLE III-9. MALES: INTERVAL MORTALITY RATES

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/l	rate	d/l	rate	d/l	rate	d/l	rate
40-30	0/86	0.000	0/50	0.000	0/50	0.000	1/50	0.020
31-210	0/86	0.000	0/50	0.000	0/50	0.000	0/49	0.000
211-240	0/86	0.000	1/50	0.020	0/50	0.000	0/49	0.000
241-270	0/86	0.000	1/49	0.020	0/50	0.000	0/49	0.000
271-300	0/86	0.000	0/48	0.000	0/50	0.000	0/49	0.000
301-330	2/86	0.023	0/48	0.000	0/50	0.000	0/49	0.000
331-360	3/84	0.036	2/48	0.042	0/50	0.000	0/49	0.000
391-420	3/80	0.038	3/44	0.068	2/50	0.040	1/49	0.020
421-450	2/77	0.026	0/41	0.000	5/48	0.104	5/48	0.104
451-480	3/75	0.040	1/41	0.024	4/43	0.093	0/43	0.000
481-510	2/72	0.028	4/40	0.100	3/39	0.077	5/43	0.116

(continued on following page)

TABLE III-9. (continued)

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/l	rate	d/l	rate	d/l	rate	d/l	rate
511-540	5/70	0.071	3/36	0.083	3/36	0.083	10/38	0.263
541-570	6/65	0.092	5/33	0.152	6/33	0.182	3/28	0.107
571-600	9/59	0.153	1/28	0.036	4/27	0.148	3/25	0.120
601-630	6/50	0.120	8/27	0.296	7/23	0.304	2/22	0.091
631-660	8/44	0.182	6/19	0.316	4/16	0.250	4/20	0.200
661-690	10/36	0.278	2/13	0.154	4/12	0.333	3/16	0.188
691-720	7/26	0.269	3/11	0.273	2/8	0.250	1/13	0.077
721-726	4/19	0.211	3/8	0.375	2/6	0.333	1/12	0.083
Terminal Kill	15		5		4		11	

corrected for continuity for combined interval:

421-510	7/77	vs	5/41(x^2 = 0.04, n.s.)	12/48(x^2 = 4.63, P < 0.05)	10/48(x^2 = 2.54, n.s.)
451-540	10/72	vs	8/41(x^2 = 0.37, n.s.)	10/43(x^2 = 1.27, n.s.)	15/43(x^2 = 6.37, P < 0.025)
481-570	13/72	vs	12/40(x^2 = 1.48, n.s.)	12/39(x^2 = 1.67, n.s.)	18/43(x^2 = 6.59, P < 0.025)
511-600	20/70	vs	9/36(x^2 = 0.03, n.s.)	13/36(x^2 = 0.32, n.s.)	16/38(x^2 = 1/47, n.s.)

TABLE III-10. FEMALES: INTERVAL MORTALITY RATES

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/l	rate	d/l	rate	d/l	rate	d/l	rate
0-150	0/86	0.000	0/50	0.000	0/50	0.000	0/50	0.000
151-180	1/86	0.012	0/50	0.000	0/50	0.000	0/50	0.000
181-240	0/85	0.000	0/50	0.000	0/50	0.000	0/50	0.000
241-270	0/85	0.000	1/50	0.020	0/50	0.000	0/50	0.000
271-300	0/85	0.000	1/49	0.020	1/50	0.020	0/50	0.000
301-330	0/85	0.000	2/48	0.042	0/49	0.000	0/50	0.000
331-360	0/85	0.000	4/46	0.087	1/49	0.020	2/50	0.040
361-390	2/85	0.024	2/42	0.048	0/48	0.000	0/48	0.000
391-420	0/83	0.000	3/40	0.075	2/48	0.042	1/48	0.021
421-450	3/83	0.036	1/37	0.027	2/46	0.044	2/47	0.043
451-480	5/80	0.063	2/36	0.056	3/44	0.068	1/45	0.022
481-510	2/75	0.027	3/34	0.088	0/41	0.000	3/44	0.068
511-540	3/73	0.041	3/31	0.097	1/41	0.024	2/41	0.049

(continued on following page)

TABLE III-10. (continued)

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/l	rate	d/l	rate	d/l	rate	d/l	rate
541-570	6/70	0.086	6/28	0.214	5/40	0.125	6/39	0.154
571-600	8/64	0.125	2/22	0.091	3/35	0.086	1/33	0.030
601-630	5/56	0.089	3/20	0.150	5/32	0.156	4/32	0.125
631-660	15/51	0.294	8/17	0.471	7/27	0.259	4/28	0.143
661-690	5/36	0.139	2/9	0.222	3/20	0.150	3/24	0.125
691-720	6/31	0.194	1/7	0.143	3/17	0.177	4/21	0.191
721-726	0/25	0.000	2/6	0.333	0/14	0.000	1/17	0.059
Terminal Kill	25		4		14		16	
corrected for continuity for combinned interval:								
421-510	10/83	vs	6/37(x^2 = 0.11, n.s.)		5/46(x^2 = 0.0, n.s.)		6/47(x^2 = 0.0, n.s.)	
451-540	10/80	vs	8/36(x^2 = 1.13, n.s.)		4/44(x^2 = 0.8. n.s.)		6/45(x^2 = 0.01, n.s.)	
481-570	11/75	vs	12/34(x^2 = 4.80, P < 0.05)		6/41(x^2 = 0.0, n.s.)		11/44(x^2 = 1.34, n.s.)	
510-600	17/73	vs	11/31(x^2 = 1.08, n.s.)		9/41(x^2 = 0.0, n.s.)		9/41(x^2 = 0.0, n.s.)	

APPENDIX B

PATHOLOGIC EVALUATIONS OF SELECTED TISSUES FROM THE DOW CHEMICAL TCDD & 2,4,5-T RAT STUDIES

Submitted to

Cancer Assessment Group

The Environmental Protection Agency

Washington, DC 20460

August 15, 1980

by

Robert A. Squire Associates, Inc.

1515 LaBelle Avenue

Ruxton, Maryland 21204

DOW TCDD CHRONIC TOXICITY STUDY IN RATS
TUMOR INCIDENCE SUMMARY TABLE

Daily Dose:	0 MCG/KG		.001 MCG/KG		.010 MCG/KG		100 MCG/KG	
Sex:	M	F	M	F	M	F	M	F
PRIMARY LUNG NEOPLASMS*	(85)	(86)	(50)	(50)	(50)	(49)	(48)	(47)
Bronchoalveolar Adenoma	2						1	
Squamous Cell Carcinoma							1	8
Bronchoalveolar Adenocarcinoma						1		
PRIMARY NASAL TURBINATE/ HARD PALATE NEOPLASMS*	(55)	(54)	(34)	(30)	(26)	(27)	(30)	(22)
Squamous Cell Carcinoma			1			1	6	5
Other: Odontoma, Tooth	1							
PRIMARY TONGUE NEOPLASMS*	(77)	(76)	(44)	(4)	(49)	(8)	(44)	(41)
Squamous Cell Carcinoma		1	1		1		3	2
Fibrosarcoma	1							
PRIMARY LIVER NEOPLASMS*	(85)	(86)	(50)	(50)	(50)	(50)	(50)	(47)
Neoplastic Nodule	4	16		8	2	26	2	23
Hepatocellular Carcinoma	2					1		10

* Number of Animals with Tissue examined Microscopically

DOW 2,4,5-T CHRONIC TOXICITY STUDY IN MALE RATS

TUMOR INCIDENCE SUMMARY TABLE

	CONTROL LEVEL	HIGH DOSE LEVEL
INTEGUMENTARY SYSTEM		
Skin/Subcutis:		
Fibroma	5/86	3/50
Carcinoma	1/86	1/50
Lipsarcoma		1/50
Malignant Fibrous Histiocytoma	2/86	
Calcifying Epithelioma	1/86	
Squamous Cell Papilloma	2/86	1/50
Squamous Cell Carcinoma	1/86	
HEMATOPOIETIC SYSTEM		
Lymph node:		
Carcinoma, metastatic		1/50
Lymphoma		1/50
Malignant Schwannoma, metastatic	1/86	
C-cell Carcinoma, metastatic	1/86	
Thymus:		
Malignant Schwannoma, metastatic	1/51	
Spleen:		
Lymphoma	1/86	
Multi sites:		
Lymphoma	2/86	
CIRCULATORY SYSTEM		
Heart:		
Endocardial Sarcoma		1/50

DOW 2,4,5-T CHRONIC TOXICITY STUDY IN MALE RATS

TUMOR INCIDENCE SUMMARY TABLE

	CONTROL LEVEL	HIGH DOSE LEVEL
DIGESTIVE SYSTEM		
Liver:		
Neoplastic Nodule	2/86	1/50
Hepatocellular Carcinoma	3/86	1/50
Pancreas:		
Acinar Adenoma	23/86	13/50
Acinar Carcinoma	1/86	
Islet Adenoma	10/86	5/50
Islet Carcinoma	1/86	1/50
Intestines(small):		
Lymphoma	2/86	
Adenocarcinoma	2/86	
Sarcoma		2/50
Intestines(large):		
Lymphoma		1/50
Tongue:		
Squamous Cell Carcinoma	1/83	5/48
Salivary Gland		
Carcinoma	1/80	
URINARY SYSTEM		
Kidney:		
Adenocarcinoma	1/86	
Tubular Carcinoma		1/50
Urinary Bladder		
Transitional Cell Papilloma	1/86	

DOW 2,4,5-T CHRONIC TOXICITY STUDY IN MALE RATS

TUMOR INCIDENCE SUMMARY TABLE

	CONTROL LEVEL	HIGH DOSE LEVEL
ENDOCRINE SYSTEM		
Pituitary		
Chromophobe Adenoma	15/80	9/49
Chromophobe Carcinoma	7/80	2/49
Adrenal:		
Pheochromocytoma	37/84	19/49
Cortical Adenoma	8/84	7/49
Cortical Carcinoma	1/84	
Ganglioneuroma		1/49
Thyroid:		
C-cell Adenoma	4/85	6/47
C-cell Carcinoma	2/85	
Parathyroid:		
Chief Cell Adenoma		1/43
REPRODUCTIVE SYSTEM		
Testes:		
Interstitial Cell Tumor	2/86	
Mammary Gland:		
Adenocarcinoma		1/50
Fibroadenoma	1/86	1/50
NERVOUS SYSTEM		
Brain:		
Astrocytoma	1/86	1/50
Granular Cell Tumor		1/50
Cranial Nerve:		
Schwannoma	1/86	

DOW 2,4,5-T CHRONIC TOXICITY STUDY IN MALE RATS

TUMOR INCIDENCE SUMMARY TABLE

	CONTROL LEVEL	HIGH DOSE LEVEL
SPECIAL SENSES		
EAR:		
Zymbals' Gland:		
Sebaceous Carcinoma	1/86	
Squamous Cell Carcinoma	3/86	1/50
EYE:		
Squamous Cell Carcinoma		1/46
MUSCULOSKELETAL SYSTEM		
BONE:		
Rib:		
Chondroma	1/86	
BODY CAVITIES		
Mesentery:		
Lipoma	1/86	
Mediastinum:		
Malignant Schwannoma	1/86	

ROBERT A. SQUIRE ASSOCIATES, INC.
1515 Lobelle Avenue
Rockville, Maryland 21204
(301) 821-0054

August 25, 1980

Dr. Bernard Haberman
Cancer Assessment Group
Office of Health and Environmental
Assessment
U.S. Environmental Protection Agency
Washington, DC 20460

Dear Dr. Haberman:

As per our agreement, we examined tissues from only the control and high dose animals from the Dow 2,4,5-T two year rat study. Since finding the one additional carcinoma in the tongue of the high dose male, however, I did examine tongues from all males in all dose groups in which there were any pathologic alterations reported by Dow pathologists. My findings agreed with those of Dow pathologists in that I found no additional neoplasms among the slides examined.

Sincerely,

Robert A. Squire

Robert A. Squire, D.V.M., Ph.D.

cc: Richard Bosof

RAS/ek

APPENDIX C

LABORATORIUM FUR PHARMAKOLOGIE UND TOXIKOLOGIE
PROFESSOR DR. F. LEUSCHNER

COPY

D-2104 Hamburg 92, January 17th, 1980

Mr.J.Guy Gwynne
Consul
Amerikanisches Generalkonsulat
Handelsabteilung
Alsterufer 27
D-2000 Hamburg 36

Dear Mr. Gwynne,

today I am allowed to answer to the questions which arose with the telex from EPA, referring to 'The Chronic Oral Toxicity of 2,4,5-T, batch No. 403, control No. 1535746 - called for short '2,4,5-T' - in Sprague-Dawley (SIV 50) Rats with special attention to Carcinogenic Properties' as follows:

A)	2,4,5-T (untreated rats)		2,4,5-T (acetone-treated rats)
	fibroma (thorax)	1 female	none
	fibroma (abdomen)	2 males 1 female	none
	fibroma (uterus)	none	1
	fibroma (mamma)	none	1 female
	fibroma (limb)	none	1 male
	interstitial cell tumour = testes	22 animals	6 animals

A1 - A4) Historical (untreated control rats, no further experience with acetone-treated animals; all historical studies 2 to 3 years before examinations with 2,4,5-T)

A1)	adenofibroma (mamma)	6 of 50 females
	interstitial cell tumour (testes)	20 of 50 animals
A2)	fibroma (limb)	3 males and 1 females of each 90 animals
	interstitial cell tumour	24 of 90 animals

- 2 -

ANSCHRIFT: FRANCOPER STR. 66b · D-2104 HAMBURG 92 (NEUGRABEN) · TELEFON: (040) 701 50 21 - 23 · 796 25 25
EXPRESSGUTSTATION: HAMBURG-HARBURG
BANKKONTO: HAMBURGER SPARKASSE (BLZ 200 505 50) · KONTO-NR. 1087/210 223

A3)	fibroma (limb)	1 male and 1 female of 50 animals each
	fibroma (ovary)	1 of 50 animals
	interstitial cell tumour (testes)	17 of 50 animals
A4)	fibroma (ovary)	1 of 100 animals
	fibroma (mamma)	1 of 100 females
	fibroma (abdomen)	3 males and 1 female of 100 animals each
	fibroma (limb)	2 of 100 males
	fibroma (head region)	1 of 100 males
	adenofibroma (mamma)	6 of 100 females
	interstitial cell tumour (testes)	32 of 100 animals

Altogether tumour rates (tumour-bearing animals) for the studies mentioned:

A)	80% males	80% females
A1)	66% males	60% females
A2)	77% males	67% females
A3)	64% males	66% females
A4)	69% males	71% females

Approximate age of the animals at the begin of the studies and duration:

A) born February, 1976
A1) born November/December, 1974
A2) born November/December, 1974
A3) born March/April, 1975
A4) born February/March, 1975

After 5 to 7 lactation weeks or quarantine start of treatment; duration of study 130 to 132 weeks (30 to 30.5 months).

B) From Group (III) 3 mg 2,4,5-T/kg b.w. only the prematurely deceased/killed animals were examined histologically. Fibromas were found in each 1 male and female (limb and abdomen respectively), interstitial cell tumours of testes in 5 rats.

C) After chronic examinations with Sprague-Dawley rats, performed by the sponsor (personal information) the highest tested dose-level (30 mg/kg) was the even subtoxic one. The dosage was fixed by the sponsor.

D) The tongue was examined macroscopically together with larynx and pharynx. These investigations did not show pathological changes therefore no histological examinations were carried out. Striated muscular tissue was taken from skeletal muscle.

E) The diet was analyzed for 2,4,5-T-stability at 6 dates and the results were as follows:

Date	Dosage mg/kg b.w.	Nominal value mg/kg standardised diet	Actual value mg/kg standardised diet
19.07.76	3	32	33
	10	112	115
	30	299	340
30.11.77	3	47	45.6
	10	165	167.4
	30	480	496.0
6.03.78	3	48	42.9
	10	168	152.6
	30	480	435.5
29.05.78	3	48	47.8
	10	163	168.2
	30	460	440.1
30.08.78	3	48	45.8
	10	160	139.3
	30	480	434.9
25.10.78	3	48	48.7
	10	160	164.0
	30	480	516.0

F) Mortality rates 2,4,5-T (mean value of males plus females)

untreated rats = 75% acetone-treated rats = 71%

F1-F4) Historical Mortality rates (F1-F4 = analogue to A1-A4)

untreated rats

F1) 71%
F2) 64%
F3) 75%
F4) 70%

G) The authors will give the permittance for these examinations. Please ask the sponsor for his agreement, this is not yet at hand.

We hope that you got complete informations on all points out of the telex of EPA and remain at your disposal for further informations.
With kind regards

APPENDIX D

LABORATORIUM FUR PHARMAKOLOGIE UND TOXIKOLOGIE
PROFESSOR DR. F. LEUSCHNER

HISTOPATHOLOGICAL EXAMINATIONS IN THE TONGUE

Appendix to
'Chronic oral Toxicity of 2,4,5-T, batch no. 503,
control no. 153574 b - called "2,4,5-T" - in
Sprague-Dawley(SIV 50) rats'
(date of final report: April 9th, 1979)

- with special attention to carcinogenic properties

Senior Pathologist:
Prof.Dr.med.W.Dontenwill

August 6th, 1980

BRAMFELDER STR. 66b · D-2104 HAMBURG 92 (NEUGRABEN) · TELEFON: (040) 701 50 21 - 23 · 796 25 25

1. REPORT ON HISTOPATHOLOGICAL EXAMINATIONS OF THE TONGUE

1.1. General informations:

The test compound 2,4,5-T was examined in 360 Sprague-Dawley (SIV 50) rats for neoplastigenic properties over 130 weeks at oral administration. Each 120 further animals served as controls with acetone as premix to the food or without any premix to the food (see final report April 9th, 1979).

1.2. Conduct of this additional study:

On August 1st, 1980 the Environmental Protection Agency asked as a first step for additional histopathological examinations in the tongue of the male rats, treated with the highest 2,4,5-T dose-level of 30 mg/kg b.w./day and of the untreated male control animals (without any premix). Therefore longitudinal sections should be prepared and investigated. On 6th of August, 1980 a further advice of the EPA asked for cross sections of the organ mentioned but at this date already longitudinal sections were taken. By this fact cross sections of the tongue in the males at 30 mg 2,4,5-T/kg b.w. and the untreated control males could not be performed.

1.3. Method:

The tongues of the male rats treated with 30 mg 2,4,5-T/kg b.w./day in the food and those of the untreated male rats (without premix) were investigated histopathologically after haematoxylin-eosin staining. Therefore longitudinal sections reaching from the retrolingual region to the tip of the tongue were prepared. Each 8 gradual sections of the tongue were investigated. The mucosal epithelial thickness of the treated and untreated rats was compared.

1.4. Findings:

The histopathological investigations in the tongue of rats showed a localised chronic mucosal inflammation with round-cell infiltration and proliferation of the connective tissue, whereby the epithelium showed above the inflammation a moderate acanthosis in the male rat no. 37, treated with 30 mg 2,4,5-T/kg b.w./day in the food. The male animal no. 44 at this dose-level had a severe phlegmonous inflammation of the tongue's musculature with small mucosal epithelial ulcers.

Apart from these two findings no changes could be seen. The variation of the epithelial thickness was, as normal, more marked at the basis of the tongue. A semiquantitative comparison did not show signs for demonstrated hyperplasia. No dysplasia, papilloma or carcinoma were found.

TABLE Histopathological Investigations in the Tongue of Rats

Animal No.	F i n d i n g s
	Group (I) Control - males -
1 - 50	no pathological findings
	Group (V) 30 mg 2,4,5-T/kg - males -
1 - 36	no pathological findings
37	localised chronic inflammation of the mucosa with round-cell infiltration and proliferation of the connective tissue, the epithelium showed above the inflammation a moderate acanthosis
38 - 43	no pathological findings
44	severe phlegmonous inflammation of the musculature with mucosal epithelial ulcers (small)
45 - 50	no pathological findings

LABORATORIUM FÜR PHARMAKOLOGIE UND TOXIKOLOGIE
PROFESSOR DR. F. LEUSCHNER

QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the data for the

'Histopathological Examination in the Tongue'
Appendix to: Chronic oral Toxicity of 2,4,5-T,
batch no. 503, control. no. 153574 b - called
"2,4,5-T" - in Sprague-Dawley(SIV 50) rats
(date of final report: April 9th, 1979)
- with special attention to carcinogenic properties -

Approved and submitted by: Franz Hübscher

August 8th, 1980

Franz Hübscher
Director of QAU

Date

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
RESEARCH AND DEVELOPMENT

SUBJECT: Clarification of Telephone Convservation with Dr. Leuschner

FROM: Wade Richardson - Wade T. Richardson
Office of Health and Environmental Assessment (RD-689)

TO: Charalingayya Hiremath, Ph.D.
Carcinogen Assessment Group (RD-689)

In early August, at CAG's request, I made an overseas telephone call to Dr. Leuschner in Germany and asked if he would be willing to cut histological sections of the tongues from male rats in his two year chronic toxicity study on 2,4,5-T. I first indicated that the Agency preferred that horizontal sections be cut. However, when Dr. Leuschner expressed preference to cut longitudinal sections, I indicated to him that I would again discuss with the appropriate people in the Agency how they felt the sections should be cut and then call him back to confirm the nature of the Agency's request. Due to some misunderstanding, it appears that longitudinal sections had already been cut by the time I called Dr. Leuschner back confirming the Agency's wish that horizontal sections be cut.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE September 12, 1980

SUBJECT Exposure Assessment for 2,4,5-T, Silvex and TCDd

FROM Acting Chief, Environmental Fate Branch, HED

TO Elizabeth Anderson
Carcinogen Assessment Group (RD-683)

Attached is the Exposure Assessment for 2,4,5-T, silvex and TCDD.

David J. Severn, Ph.D.

cc: P. E. McGrath

QUANTITATIVE ASSESSMENT OF EXPOSURE TO 2,4,5-T, SILVEX AND TCDD

September 12, 1980

QUANTITATIVE ASSESSMENT OF EXPOSURE TO 2,4,5-T, SILVEX AND TCDD

INTRODUCTION

As part of its risk-benefit balancing procedures, the Agency generally attempts to estimate potential human exposure to pesticides in quantitative terms. The ultimate objective of these assessments is to develop numerical estimates of the amount of exposure that certain segments of the population may experience as a result of pesticide use. These exposure data are combined with toxicity information to generate an overall risk assessment. The risk assessments are then used to predict potential health effects based on the toxicologic effects of the pesticide in question.

This document provides some quantitative estimates of exposure to 2,4,5-T, silvex, and TCDD for use in the cancellation hearings. These estimates are based as far as possible on observed residue levels in the environment. However, while these estimates are expressed as numerical values, they are in fact much less precise than their numerical nature would imply. This is because the available data are meager, because conditions (spray techniques, weather, etc.) are so variable, and because many assumptions have to be utilized in order to arrive at the estimates. This introduction describes some of the reservations which apply to the numerical estimates presented in this assessment, and comments on the limitations on the use and interpretations of this information.

General

Agency exposure assessments, including this analysis for 2,4,5-T, silvex, and TCDD, are based where possible on actual field data. In the present case, the data upon which this exposure assessment is based include data on chemical residues in soil, food and other environmental materials, on actual field exposure data for applicators, and on the data on transport and fate of these chemicals in the environment.

In addition, information on pesticide use practices and extent of use is necessary to arrive at reasonable estimates of exposure. This information includes the crops or sites which may be treated, the rates and methods of application, and information on the other activities during their subsequent application. This information is used to develop estimates of the number of people potentially exposed to the chemicals by oral, dermal and inhalation routes as a result of specific use practices.

The information available for use in this exposure assessment is variable as to its completeness, quality, and reliability. In general, the greatest confidence can be placed on the field exposure and residue data, even though it is incomplete in many ways. The information relating to use practices is somewhat less certain. Agency scientists started with information from the pesticide label to determine application rates and crops or sites likely to be treated. Estimates relating to the extent of sites or crops

treated and other indicators of the probable extent of contamination are subject to many uncertainties. In particular, the numerical values for the populations at risk are highly uncertain. This is because information on population demographics whether or not related to pesticide use, is not well developed.

The uncertainties described above are common, in varying degrees, to all exposure assessments, including these assessments for 2,4,5-T, silvex and TCDD. In sum, although Agency scientists have a high degree of confidence about much of the empirical data which form the basis for this analysis, they are far less confident about other information. The quantitative exposure estimates for the populations at risk are limited by these uncertainties.

Exposure Analysis

The starting point for exposure assessment for pesticides is descriptive information on pesticide release and distribution to the different environmental compartments such as air, water, soil, and animal and plant tissues during application. In addition, 2,4,5-T and silvex are known to move from sites of application to non-target areas under some conditions of application.

This qualitative information on potential sources of human exposure is supported by analytical chemical data showing that residues of these chemicals are present subsequent to application,

both a application sites and at non-target sites. Such chemical residue information provides the initial numerical base for quantitative estimates of possible human exposure. For example, unlike many pesticides with relatively short half-lives and relatively rapid disappearance from the environment, 2,4,5-T and silvex may persist in the environment for several months after application; TCDD may remain for several months or years. Therefore, special concern is raised about 2,4,5-T, silvex and TCDD because they may remain in the environment in significant concentrations for several months or years after their application.

However, despite the availability of some useful information, there are gaps in our knowledge. For example, although large amounts of 2,4,5-T and silvex are used each year, comprehensive monitoring information on 2,4,5-T, silvex, and TCDD residues in the environment is, for the most part, unavailable.*/ This paucity of residue information limits the Agency's ability to make quantitative exposure estimates to only some routes of exposure and only for certain uses.

*/ The paucity of monitoring data on TCDD is due largely to the only recent development of analytical methodologies with sufficient sensitivity to measure the extremely low levels of TCDD which are of biological concern, to the limited number of facilities with these analytical capabilities, and to the high cost of analyzing samples at these levels. For 2,4,5-T and silvex, the problem of insufficient monitoring information appears to be largely due to a lack of comprehensive monitoring programs, or inappropriate sampling.

Even when some data are available for one kind of application, there may be uncertainty as to whether those data are applicable to other applications which may occur under different conditions. For example, residue data collected during springtime application in the Pacific Northwest may not properly describe the amount and distribution of chemicals under different environmental conditions at a different time of the year. Often, the only data available are data derived from laboratory studies, with little or no field data to verify that the laboratory data accurately describe the residue levels which might be present under field conditions.

Further, each of the several different human exposure pathways provides a different kind of exposure potential. Even when some empirical residue data on a given route of exposure are available, there are often uncertainties concerning the generalization of those data to other routes of exposure. These uncertainties are a particular concern when estimating exposure to chemicals such as TCDD which appear to pose risks at very low levels of exposure.

In attempting to generalize to "average" or "typical" use patterns, the Agency has encountered a wide variety of practices, which were very difficult to address. An example is the application rate to be used when rangeland vegetation is spot treated. Despite the fact that the USDA-EPA States Report (Ref. 2) notes a

2 lb/A maximal application rate on grazing lands, it was found that other rates have been used and are permitted by the label. Also, despite "typical" 5-15 year recommended intervals between herbicide spray applications, instances of successive annual treatments have been substantiated, and may, in fact, be more a common practice than the USDA Report assumes.

A very difficult aspect of quantitating risk is specifically identifying and quantitating populations at risk. The Agency has found, for example, that deer and elk from 2,4,5-T treated forested areas may contain TCDD residues in their fat at readily measured levels. Also, it is known that some people include deer and elk in their diets. But, the proportion of deer and elk taken by hunters annually that are actually contaminated, the level of contamination, and the numbers of people who consume given amounts of contaminated meat is not known.

To extrapolate from the available information to potential human exposure (and subsequently to risk assessments), assumptions based on the observed residue data, information about use practices, and "typical" consumption patterns are made. These assumptions may either over- or under-estimate actual risk. This can be confirmed only by the acquisition of additional data. Nevertheless, the Agency has developed some numerical values, however uncertain, to permit the quantitative estimation of risk for the cancellation proceedings.

The exposures which have been quantified in this document are as follows:**/

1) Occupational exposure to 2,4,5-T, silvex, and TCDD.

2) Dietary exposure of the general population and local populations to TCDD residues in beef and local populations to TCDD residues in dairy products resulting from the use of 2,4,5-T and silvex on rangeland and pasture.

3) Dietary exposure of local populations to TCDD residues in deer and elk resulting from the forestry use of 2,4,5-T and silvex.

4) Dietary exposure of the general population and local population to silvex residues in rice, apples, pears, prunes, and sugar (from sugarcane) resulting from the use of silvex on these food products.

5) Dietary exposure of the general population and local populations to 2,4,5-T and/or silvex residues in rice resulting from the use of 2,4,5-T and silvex on rice.

Finally, the available data relating to some uses of 2,4,5-T and silvex are inadequate even to begin assessing potential human exposure. For some situations, no monitoring information is known to the Agency, and in other situations the available data

**/ The Agency is still evaluating and generating monitoring data which were not utilized in these quantitative assessments. The Agency may utilize these data as they are developed.

are too incomplete or too uncertain to provide the basis for even a simple estimate of exposure. It is emphasized that the incompleteness of data and the consequent lack of an exposure analysis mean only that suitable data were not available, not that these pathways are biologically insignificant.

ESTIMATION OF OCCUPATIONAL EXPOSURE TO 2,4,5-T, SILVEX, AND TCDD

Introduction

This analysis provides a quantitative human exposure */ estimate for 2,4,5-T, silvex, and dioxin in terms of absorption by the body of these chemicals under normal agricultural working conditions.

Human exposure estimates are made on the basis of chemical analyses of dermal and inhaled concentrations of the chemical or chemicals, and if the information is available, on the basis of the amount of chemical(s) or their metabolites excreted by the body (e.g. in the urine). **/

In the case of the pesticides and contaminant under consideration, there are experimental data available on the occupational exposure to pesticide applicators and farmworkers applying 2,4,5-T under actual use conditions. These data consist of dermal, inhalation, and urinary concentrations of 2,4,5-T obtained from the field application of 2,4,5-T in forestry and rice***. Exposures to 2,4,5,-T from other uses and to silvex and TCDD for all uses were estimated by extrapolation and will be discussed below.

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* The term "exposure", as used in this paper, refers to the amount of chemical absorbed by the body.

** During the past four years, since the initiation of the RPAR process, the Hazard Evaluation Division has estimated occupational exposures to many pesticides. In some cases data on dermal and inhalation exposure were available for these estimates. In other cases, these data had not been generated, necessitating extrapolations from information on other pesticides (with similar application techniques) for purposes of the exposure estimate.

*** Experimental data of the type required for this analysis were found only for 2,4,5-T. Consequently, exposure to silvex and TCDD was calculated on the basis of extrapolations from the 2,4,5-T data as explained in the text.

Duration of exposure to specified occupational groups and the number of individuals comprising these groups are critical elements in risk assessment. These parameters were estimated from use data from Reference 2 and are summarized in the Appendix (page 48, et seq.) Occupational exposure to 2,4,5-T, silvex, and TCDD are estimated for the following uses:

- forestry
- rice
- range and pasture
- rights-of-way

It should be noted that because of information gaps, it was necessary to make a number of assumptions and extrapolations in estimating applicator exposure to 2,4,5-T, silvex, and TCDD. As a result, our estimates are subject to a considerable degree of uncertainty.

Estimation of Occupational Exposure to 2,4,5-T

We are aware of three studies on the exposure of applicators to 2,4,5-T which provide experimental data to be used for exposure assessment. The most detailed of these studies is one conducted by Lavy on forest applicators (Ref. 14, 15). The data from this study has been analyzed using a pharmacokinetic model in a report by Ramsey et al. (Ref. 19). Lavy also conducted a somewhat abbreviated study of workers applying 2,4,5-T to rice and forests (Ref. 16). The third study yielding useful exposure information is one by Kolmodin-Hedman et al. (Ref. 13) in which two professional tractor crews consisting of two persons each were monitored for 2,4,5-T during and after two applications of 2,4,5-T to forests.

Two other studies reported in the literature */ provided confirmatory information on 2,4,5-T absorption by humans.

The information enabling us to estimate the absorption of 2,4,5-T by occupationally exposed individuals is contained in the field study conducted by Lavy on foresty applicators (Refs.14,15). The study was designed to measure 2,4,5-T exposure to pesticide workers applying this pesticide in the forest by three different methods:

- aerial (helicopter)
- ground application by tractor-driven mist blower
- ground application by backpack sprayers

Twenty-one individuals (including two females) participated in this study. The subjects were engaged in normal pesticide application activities (e.g. piloting a helicopter; driving a tractor and handling pesticide application equipment; mixing pesticides by dilution, etc.) A commercial product containing 2,4,5-T Esteron[9], was applied at day "0" at a rate of 2 lbs a.e./A*

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* Shafik et al. (Ref.24) report an average of 2.4 mg 2,4,5-T/l of urine in 6 spray operators engaged in 2,4,5-T application. No spray history or total excretion is given, so it is impossible to calculate total exposure from this experiment. As a matter of fact, the purpose of the reported study was to develop analytical methodology rather than measure exposure.

Simpson et al. (Ref.25), in a very brief summary paper, reported urinary levels of 2,4,5-T in pesticide applicators handling this herbicide ranging from 0.160 mg/l to 1.740 mg/l. These incomplete results make it impossible to calculate total body burden from 2,4,5-T exposure.

* a.e. = acid equivalent

for tractor-driven mist blower and helicopter applications and 1.6 lbs./A in the backpack study. Urinalyses for 2,4,5-T (acid) were performed daily for 7 days including 1 sample prior to exposure. On the 7th day, the herbicide application was repeated by the same individuals, and urine samples were analyzed as before. Dermal absorption was measured by the use of cellulose-backed gauze patches which were placed according to directions given by Wolfe, et al. (Ref.31).

Typical attire of individuals participating in the study was long trousers, shirt (long or short sleeves), cloth sneakers, and leather or field boots. Temperatures during the experiment ranged from a low of 18°C to a high of 26°C. Wind speeds on 5 days of application were recorded at 0 mph while the wind speed ranged from 0-5 mph on three other days. The experiments were carried out in South Central Arkansas near Hot Springs, Hampton, and New Monticello. The terrain there is less hilly than other areas where 2,4,5-T and silvex are used, such as that in western Washington and Oregon. It is conceivable that different terrain and weather conditions may change the exposure pattern of the occupationally exposed population. However, we know of no experimental work that has been carried out to investigate these variations. Complete experimental details may be found in the Project Completion Report (Ref.14) and in the published paper (Ref.15).

According to Ramsey et al. (ref.19), "the total amount of 2,4,5-T excreted in the urine following exposure represents a minimum estimate of the amount

...absorbed, since urinary excretion may not be complete at termination of the experiment. However, calculation of the absorbed dose of 2,4,5-T based on pharmacokinetic analysis... is not dependent on total excretion and can, therefore, provide a more realistic estimate of the absorbed dose." Ramsey et al. have chosen maximum estimated doses of 2,4,5-T obtained from three different kinetic equations (Ref.19, p. 20).

We have used Ramsey's adjusted data based on Lavy's study (Refs.14,15) in estimating occupational exposure. Results for forestry application of 2,4,5-T are tabulated in the last column of Table 1, giving the average experimental dose expressed as mg/kg body weight/hour. From Tables 2-A and 3-A it may be seen that some individual values varied widely. For example, the ranges for pilots were 0.005 - 0.024 mg/kg/hour and backpack applicators, 0.009 - 0.036 mg/kg/hour.

Lavy (Refs.14,15) provides experimental data only for forestry uses of 2,4,5-T. Therefore, exposure estimates for uses on rice, rangeland, pasture, and rights-of-way were calculated by comparing application rates, occupations, and application techniques with the corresponding figures in forestry use, assuming that exposure would be directly proportional to the application rate. It was further assumed that the difference in application rate was the only variable factor which would result in differences of applicator exposure for each type of occupational group. For example, the rate used for aerial application of 2,4,5-T in range and pasture is

1 lb/A (weighted average) and the corresponding rate in forest is 2.0 lbs/A (average). Thus, the exposure values for different occupational groups for range and pasture use is estimated by multiplying the experimental value (forestry use) by one-half.*

In order to convert unit exposure values to dose/person/hour, the figure in the last column of Table 1 may be multiplied by the estimated average body weight of a male worker, namely 70 kg. Table 1 also provides data on the estimated annual hours of exposure to each occupational group of workers and estimated number of workers in each occupational category. These numbers were derived from the total acreage** treated, found in Reference 2. The methodologies for arriving at these estimates are fully explained in the Appendix.

In the Lavy study (Refs.14,15), dermal and inhalation exposures by field personnel were measured. In addition, urinary 2,4,5-T and other urine

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* Confirmation that absorption, as measured by urinary excretion, is directly proportional to dose applied has been recently shown by Franklin, et al. in a study involving the insecticide azinophosmethyl and orchard workers (soon to be published) (C.A., Franklin, R.A. Fenske, R. Greenhalgh, L. Mathieu, H.V. Denley, J.T., Leffingwell, and R.C. Spear, A Comparison of Direct and Indirect Methods of Estimating Dermal Exposure to Guthion in Orchard Workers. Accepted for publication in J. Toxicol. Env. Health).

** Reference 2 apparently does not separate 2,4,5-T and silvex treatment for range and pastures, although this is not explicitly stated. Since under recent usage pattern, silvex represents only 10% (Ref. 35) of the combined use of 2,4,5-T and silvex, we feel that our estimates of annual hours of exposure and number of workers in each exposed occupational group are indeed representative of 2,4,5-T treatment alone without correcting for the small percentage of silvex.

TABLE 1

Estimated Exposure of Pesticide Applicators and Farmworkers to 2,4,5-T

Use Pattern	Exposed Group	Application Rate[1] (lb/A)	Estimated No. Exposed Persons[1]	Estimated Exposure (hrs/yr)	Average Exposure[2] (mg/kg/hr)
FORESTRY					
1. Aerial	Pilots	2	73	200	0.015
	Mixer/Loaders	2	73-145	800	0.062
	Flaggers	2	—[3]	800	0.003
	Supervisors	2	—[3]	800	0.004
2. Ground Broadcast					
a. Tractor Mistblower	Mixer/Loader	2	90-180	480	0.020
	Tractor/operator/worker	2	90	240	0.013
	Supervisor	2	—[3]	480	0.006
b. Backpack Sprayer	Applicators	1.6	300	800	0.021
	Mixer/Supervisor	1.6	—[3]	800	0.005
RANGE AND PASTURE					
1. Aerial	Pilots	1.0	130	75	0.008[4]
	Mixer/Loaders	1.0	130-260	100	0.031[4]
	Flaggers	1.0	800	25	0.002[4]
2. Ground Backpack	Applicators	0.6	20,000	80	0.008[4]
RICE					
Aerial	Pilots	1.0	307	12	0.008[4]
	Mixer/Loader	1.0	307	48	0.030[4]
	Flaggers	1.0	6500-9500	0.6	0.002[4]
RIGHTS-OF-WAY					
1. Aerial	Pilots	8.0	25	400	0.060[4]
	Mixer/Loaders	8.0	25-50	400	0.240[4]
2. Ground					
a. Selective Basal	Applicators (hand)	6.4	1380	1000	0.084[4]
b. Cut Stump	Applicators (hand)	4.0	60	500	0.053[4]
c. Mixed Brush	Applicators (hand)	6.0	270	660	0.079[4]
	Truck boom Applicators	0.8	178	660	0.005[4]
d. Railroad	Crew of Four	5.(avg)	114	264	0.066[4]
e. Electric Power	Applicators (hand)	6.(avg)	400	660	0.080[4]

1. See Table 1-A
2. Reference 19. Calculated dose levels; received by EPA on February 14, 1979; ≒ 16P [30,000/26]; See also Table 2-A for raw data.
3. (——) indicates that the number of individuals cannot be estimated.
4. These values were extrapolated as explained in the text.

components were analyzed. By Lavy's calculations, very poor correlation existed between dermal exposure to 2,4,5-T, as measured by 2,4,5-T analyses of the body patches, and the amounts excreted in the urine.* One explanation for the lack of correlation might be the fact that the dermal exposure patches were not always placed in areas of highest potential exposure, e.g. the hands of mixer-loaders. Thus, the exposure derived from dermal patches might be expected to be too low, and, consequently, urinary excretion values would be more realistic.

In the second Lavy 2,4,5-T-exposure study (Ref.16), only dermal and no urinary analyses for 2,4,5-T were performed. However, only results from urinary excretion experiments were utilized by us for exposure estimates for the following reasons:

1. The pharmacokinetic behavior of 2,4,5-T has been described in mammals, including man.
2. Analysis of 2,4,5-T in the urine is a more direct measurement of 2,4,5-T absorption than the use of dermal patches.

Thus, in our exposure estimates for 2,4,5-T we have utilized exclusively urinary excretion data derived from Lavy's field study (Refs.14,15), transposed by pharmacokinetic calculations by Ramsey, et al. (Ref.19).

While we have relied heavily on Lavy's field studies and the pharmacokinetic derivations by Ramsey, et al., based on the same studies, it is

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* Exposure through inhalation was much lower than that from dermal contact and, therefore, was not included by Lavy in the correlation test.

prudent to review these experimental studies and kinetic derivations in greater detail. During the cross examination testimony of Dr. Nisbet, several experimental deficiencies in the Lavy studies (Refs.14,15) were discussed and included apparently incomplete or variable urine collection and failure to correct urine volumes according to creatinine levels.

The Agency is presently engaged in an independent analysis of the pharmacokinetic treatment of Lavy's field data. After this review has been completed, the exposure estimates may have to be revised appropriately.

KOLMODIN-HELMAN STUDY

Recently, another study from Sweden on the exposure of two tractor crews to 2,4,5-T has come to our attention (Ref.13). The study consisted of the surveillance of two work crews of 2 individuals each. They applied a mixture of phenoxy herbicides in a forest for one work week and 2-4 hrs/day spraying time using a Gullvik* Forest Tractor equipped with a fan sprayer. Blood and urine samples were analyzed before application of the herbicide, once or twice during the application period, and at 12, 24, and 36 hours after the last application. Urine samples were not taken at regular intervals during the study, making it less reliable for the estimation of total exposure than Lavy's study (Refs.14,15). Lavy showed that even a 6 day period is insufficient for complete elimination of 2,4,5-T from the body. Thus, it is quite certain that Kolmodin's results are on

* The make of the Swedish tractor is mentioned because the difference in exposure between Swedish and U.S. workers may be due to equipment differences.

the low side, since the last urine sample was taken only 1.5 days after the last application of 2,4,5-T. Nevertheless, we compared Kolmodin's results with Lavy's data. Table 2 recapitulates the urinalysis results originally reported by Kolmodin, et al. as well as the interpolated values on the days on which no urine sample was taken.

TABLE 2

URINARY EXCRETION OF 2,4,5-T (mg/L)†

	PERSONS			
DAY	KK**	LJ**	JG**	LEO**
Monday	0.5***	0.5	3.1	1.3
Tuesday	1.0	0.4	11.4	4.9
Wednesday	1*	1*	9*	4*
Thursday	1*	1*	6.5	3.7
Friday	1.2	1.2	4.2; 3.0 (3.6 avg)	2.3; 3.3 (2.8 avg)
Saturday	0.9	0.9	2.7	4.3
Sunday (PM)	0.7; 0.4 (0.6 avg)	1.0; 0.7 (0.9 avg)	2.1; 2.2 (2.2 avg)	3.5; 2.5 (3.0 avg)
Total (mg/L)	6.2	5.9	38.5	24.0

† Reference 13.

* Interpolated; no experimental values

** KK was a mixer-worker and row leader in Crew I
LJ was a tractor driver in Crew I
JG was a tractor driver in Crew II
LEO was mixer-loader & row leader in Crew II

*** Analysis before first treatment were of the order of less than 0.05 ppm.

Exposure began on Monday and ended on Friday.

The exposure by Crew II in Kolmodin's study appears to be 3 to 6 times higher than that of Crew I. The reason for this may possibly be explained by the different working conditions during pesticide application by Crews I and II. Crew I changed work clothes each evening and their tractor had a partially protected seat. On the other hand, the mixer/worker of Crew II only changed his shirt in the middle of the week. Also, the tractor for Crew II had a completely open seat. In addition, the mixer/worker for Crew II, who also performed the job of row leader, could have received spray each time the tractor turned, as could the tractor driver, depending on the direction of the wind. Table 3 summarizes and compares the results of the exposure to 2,4,5-T of the two work crews in Kolmodin's study.

TABLE 3

EXPOSURE TO 2,4,5-T*

Crew No.	Person	Occupation	kg BW	Spray time (hrs/day)	Total mg excreted**	mg/kg-BW	mg/kg BW/hr***
I	KK	Mixer/worker	70	2-4 hours	9.30	0.13	0.01
	LJ	Tractor Driver	80	2-4 hours	8.85	0.11	0.01
II	LEO	Mixer/worker	75	2-4 hours	36.0	0.48	0.03
	JG	Tractor Driver	62	2-4 hours	57.75	0.93	0.06

Appropriate: 2-3 kg AI/ha (equivalent to about 2 lb/A) 330 g/liter 2,4-D and 170 g/liter 2,4,5-T. This calculates to about 0.66 lb./A 2,4,5-T

CREW I Jeans, shirt; changed work clothes before evening meal. Tractor has partially protected seat. The sprayed areas were marked by KK.

CREW II Jeans and shirt; LEO was the mixer and changed shirt once. JG was the tractor driver. LEO was "row leader." (A person who marks the row to direct tractor-driver). When the tractor turned, he could get spray liquid on his body. Tractor driver could also receive spray on his body, since tractor had a completely open seat.

* Reference 13.
** Based on 1.5 L urine/day; see Table 2 for tabulations.
*** Average 3x5 = 15 hrs/week spray time.

Table 4 is a comparison of the results from Tables 1 and 3

Table 4

Comparison of Lavy and Kolmoden-Hedman Studies

	Lavy Study (Refs.14,15)		Kolmodin Study (Ref.13)		
Occupation	Av. Dose (mg/kg/hr)	Applic. Rate (lbs/A)	Av. Dose (mg/kg/hr) Crew I	Crew II	Applic. Rate (lbs/A)
Mixer/Loader (ground)	0.020	2	0.01	0.03	0.66
Tractor Driver	0.013	2	0.01	0.06	0.66

By multiplying the exposure values obtained by Kolmodin by a factor of 3 (to adjust for the lower application rate in Kolmodin's study), the tractor driver of Crew II would appear to have a significantly higher exposure (by a factor of approximately 14) than the corresponding U.S. workers in the Lavy studies.

If the conditions of described by Kolmodin are typical of those encountered in the United States, it may be prudent to perform a quantitative risk assessment using the higher exposure figures.

EXPOSURE TO SILVEX AND TCDD

We could find no reports, either published or unpublished, on the exposure of workers in the field to silvex or TCDD. Therefore, in order to estimate occupational exposure to these chemicals, we have assumed the following:

1. Silvex exposure is the same as 2,4,5-T exposure, wherever and whenever the use pattern for silvex and 2,4,5-T are similar or identical. We believe that the chemical behavior of silvex and 2,4,5-T is sufficiently similar to justify this assumption.

2. We are not aware of any information regarding the rate of dermal absorption by man of TCDD relative to 2,4,5-T. In the absence of this information, we are assuming for the purpose of estimating exposure that TCDD and 2,4,5-T are absorbed at the same rate.*

3. TCDD exposure resulting from 2,4,5-T application may be estimated by applying concentration factors obtained by direct analysis of 2,4,5-T formulations. Lavy reported that TCDD was present in the Esteron® product used in his study (Refs. 14,15) at a level of 0.04 ppm (4×10^{-8}). Manufacturer's voluntary specifications of current 2,4,5-T production claim TCDD concentrations of 0.1 ppm or less.** Thus, TCDD exposure may be estimated by multiplying 2,4,5-T exposure for each applicator group by a factor ranging from 4×10^{-8} to 1×10^{-7}.***

4. Estimates for number of exposed individuals and annual hours of exposure due to silvex use can be made by using conversion factors based on ratios of 2,4,5-T treated acres to silvex treated acres for different uses as shown in Table 5; these ratios range from 1/10 to 1/1000.

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* Another assumption is that the concentration of TCDD relative to 2,4,5-T does not change from the time it is formulated until it is deposited on the skin of the occupationally exposed personnel.

** There are some manufacturers who claim that their 2,4,5-T products contain 0.02 ppm or even less dioxin.

*** Since the concentrations of TCDD in 2,4,5-T and silvex are approximately the same, the same factors may be used in estimating exposure to TCDD resulting from silvex applications. The same number of persons exposed to 2,4,5-T or silvex are, therefore, assumed to be exposed to TCDD. Moreover, the annual hours of exposure of a person to 2,4,5-T and/or silvex are assumed to be the same as his annual hours of exposure to TCDD.

Table 5

Comparison of Relative Rates of Usage of 2,4,5-T and Silvex

Uses	2,4,5-T:Silvex Ratio
Rangeland/pasture[a]	10:1
Forestry (Ref.2)	100:1
Rice[b]	1000:1
Rights-of-way[b]	appx. 10:1

a. Reference 35.
b. Reference 17.

EXPOSURE ESTIMATE - INCREASED USE OF 2,4,5-T AND SILVEX

The exposure estimates summarized in Table 1 are based on recent pre-suspension use volume data for 2,4,5-T and silvex. For all registered uses, only a relatively low percentage of all potential acreage is actually treated with these two herbicides. If the acreage treated were to increase, the total number of exposure hours * would increase proportionately. It is extremely unlikely that one hundred percent of the acreage which could be treated annually with 2,4,5-T or silvex consistent with the labeling would in fact be treated. ** However, because the increase in annual exposure hours resulting from such maximum possible use provides an upper limit on the total number of annual exposure hours, we are estimating the increase in total number of exposure hoiurs which would result from such maximum possible use.

Of the approximately one billion acres of pasture and rangeland in the U.S., only 0.33% is treated with either 2,4,5-T or silvex. If all pasture and rangeland were treated annually,** the total annual exposure hours for

*/ Total number of exposure hours is defined as the product of total number of workers in a particular occupational group times the annual number of hours per worker for this use.

**/ In fact, only 26% of total rangeland and pasture land has undesirable plants susceptible to treatment by 2,4,5-T or silvex. (Ref. 17)

each type of applicator would increase by a factor of 300 over our estimate of total number of annual exposure hours estimated to occur at the time of suspension.

Similar projections for increase in total number of exposure hours to either 2,4,5-T, silvex, or TCDD might be made if the extent of use of 2,4,5-T or silvex approached the maximum possible market for commercial forest land (factor = 500), rice land (factor of 10), or rights-of-way (factor = 200) (ref. 17).

SUMMARY OF OCCUPATIONAL EXPOSURE

Based on the Lavy study, which measured 2,4,5-T levels in the urine of applicators who applied 2,4,5-T, as well as on a pharmacokinetic analysis by Ramsey of these experimental data, we have estimated applicator exposure to 2,4,5-T, silvex and TCDD resulting from a number of uses of 2,4,5-T and silvex. These estimates are provided in Table 1.

Because of several factors, the exposure estimates made in this document are subject to considerable uncertainty. Some of the more important factors are:

1. It is possible that the degree of care to avoid exposure which was exercised by the applicators in the Lavy study may not be typical of that used in routine 2,4,5-T or silvex applications.

2. The applications in the Lavy study were conducted under essentially windless conditions and on relatively level terrain. At higher wind velocities or different terrain (rolling hills or mountains) exposure rates may be quite different

3. In estimating TCDD exposure, it was necessary to extrapolate from data on 2,4,5-T exposure. In so doing, it was assumed that TCDD was absorbed by the body with an efficiency equal to that of 2,4,5-T. In fact, TCDD may be absorbed at rates considerably different than those of 2,4,5-T.

4. The Lavy study may have had certain experimental deficiencies, including incomplete or variable urine collections.

The Swedish study (ref.13) indicated that under certain conditions, applicator exposure, at least with respect to tractor drivers, may be considerably higher than that estimated from data generated in the Lavy study. Correcting for differences in application rates, the exposure rate of one of the tractor-drivers in the Swedish study was about 14 times higher than the exposure rate measured in his American counterpart (0.18 vs. 0.013 mg/kg/hr). Thus, if U.S. field conditions were comparable to those encountered in the Swedish study, it might be prudent to estimate risk on the basis of higher levels of exposure than those found in the one U.S. study.

ESTIMATES OF HUMAN EXPOSURE TO BEEF AND MILK CONTAMINATED WITH TCDD

BACKGROUND

The estimates of human exposure to TCDD from contaminated beef and milk which are developed in this document are based on a two-part study (hereafter called phase one and phase two, respectively) initiated under the Dioxin Implementation Plan in 1975. These studies were designed to determine possible residues of TCDD in the fat and livers of cattle grazing on range land treated with 2,4,5-T (ref.26).

Animals from selected farms in Missouri, Kansas, Texas and Oklahoma were taken to commercial slaughter houses, where samples of fat and liver were collected. Along with historical information, these samples were forwarded to the Toxicant Analysis Center, at Bay St. Louis, Mississippi, for extraction, cleanup, and encoding, preparatory to chemical analysis for tetrachlorodibenzo-p-dioxin (TCDD) by various analytical collaborators (ref.26).

The phase one samples were taken in February/March, 1975, and the phase two samples in November/December, 1975, from cattle grazing on forage treated with 2,4,5-T in May, 1974 and May, 1975, respectively. In both parts of the study, the application rates varied from farm to farm, ranging from 1/2 to 4 lb 2,4,5-T active ingredient/A (3 lb/A maximum application rate in phase two). In addition, the percentage of acreage actually treated varied from 20% to 100%.

Agricultural practices appear to have been about the same as those in use today. Herbicide (2,4,5-T) was aerially applied (with occasional ground spot-treatment) to control undesirable vegetation on grazing

land. Cattle from the selected farms were routinely sent directly to slaughter (ref.26), rather than to supplementary feed lots. This practice proved useful for the subsequent TCDD analyses, since it avoided dilution of TCDD residues by the deposition of additional interstitial fat. Additional farms were selected which had no known recent treatment of chlorinated phenoxy herbicides. Adipose and liver samples from animals collected from this group of farms served as experimental controls.

The decision to search for TCDD in adipose tissues was based on the presumption that TCDD, as a lipophilic chemical, would be preferentially distributed to lipid-rich tissues and could most easily be detected there. Data on residues confirmed in such tissues could then be used to estimate levels in other tissues (such as milk or meat) which consist only partially of fat (5% and 15%, respectively). In fact, this may be the only technique available to estimate such residues, since TCDD residues in tissues such as these might well be below the limit of detection using current technology, and thus cannot be directly measured.

Because the beef studies were conducted as "field" monitoring studies, they could not be controlled as well as "laboratory" experiments. Consequently, a number of uncertainties must be noted, about which different assumptions could yield alternative interpretations of the data.

Ross (ref.22) observed that virtually all of the positive samples in phase one (3 lb/A group) came from several adjacent Missouri farms. This is not surprising, especially in light of the high application rates used on these farms (3 lbs/A).

There is also the possibility that the dioxin residues in these fat samples might not be representative of the residues in all cattle allowed to graze on 2,4,5-T-treated land. Since this study contains the most reliable field data currently available, however, it is assumed that these residues are representative of the residues which would result from typical 2,4,5-T-use on range land in the United States. Further, it is reasonable to extend the conclusions regarding 2,4,5-T use to the use of silvex on pasture land, since the use practices for the two herbicides are very similar, and both contain comparable amounts of TCDD.

Another uncertainty concerns the amount of treated vegetation actually ingested by the exposed cattle. Since the percentage of 2,4,5-T-treated grazing lands varied widely from farm to farm (from 20% to 100%), cattle might have had the opportunity of ingesting differing percentages of both treated and untreated vegetation, depending upon the grazing acreage in which they were allowed to feed. Since the exact situation on each farm is unknown, it is assumed that 100% of the diet of these cattle consisted of contaminated vegetation, that is, cattle fed selectively on the treated areas, rather than grazed indiscriminately, and consumed no supplementary (uncontaminated) feed or forage. This assumption was made because there appears to be a better correlation between average application rate and average residue levels when it is assumed that animals grazed solely on treated vegetation, rather than on both treated and untreated vegetation.

It is therefore assumed that the dietary intake of forage in the cattle from this study consisted of only treated forage. If these cattle actually ingested significant quantities of forage from untreated areas, or supplemented their diets with uncontaminated feed or grain, then it is highly

probable that any cattle which might have actually consumed exclusively contaminated vegetation would have had even higher TCDD residues in their adipose tissues than those measured in this study.

SUMMARY OF RESULTS

Collaborating analytical laboratories included an EPA contractor (Northrup Laboratory), Wright State University, the Dow Chemical Company and Harvard University. In phase two of the study, only Wright State University has analyzed the samples thus far. A second laboratory is scheduled to perform confirmatory analyses.

The results of phase one which were generated by high resolution gas chromatography/mass spectroscopy are summarized in the Table A-4. Residues were detected in 7 of 11 samples, ranging from not detected (ND), (limits of detection ranging from 3 to 24 ppt) to 89 ppt in the 3 lb/A group. The percentage of samples with detectable residues appeared to decrease with decreasing application rates. The 1/2 lb/A group yielded no samples with detectable residues, with limits of detection from 2 to 10 ppt.

The 18 control samples (reported in Table 1 of EPA Exhibit 564) were analyzed by high resolution GC/MS. Sixteen were unequivocally negative at limits of detection from 3 to 23 ppt TCDD. One control was analyzed five times, and yielded one positive value of 20 ppt (Limit of detection of 12) and four ND's (limits of detection of 5-14 ppt). The remaining control was analyzed 15 times, and yielded 4 positive values of 3,6,19 and 61 ppt (with limits of detection of 3-10 ppt) and 11 ND's (limits of detection of 1-20 ppt): Since virtually all analyses were negative, no

corrections were made to the data summarized in Tables A-4 or A-5. The preliminary results of phase two are summarized in the Table A-5. However, these data have been included for comparison only and will not be incorporated into the dietary estimate because only two samples were taken from animals grazing on land treated at the highest application rate (3 lb./acre). Residues of TCDD found in the adipose tissues of these cattle ranged from ND(limits of detection ranging from 7 to 14 ppt) to 34 ppt in the 2 lb/A group, but were all nondetected in the 3/4 lb/A group (with limits of detection of 7-14 ppt). Although of a preliminary nature, these results are of the same order of magnitude as those found in phase one.

ASSIGNED RESIDUE VALUES

Since many of the positive samples tended to occur at levels just above the limit of detection of current methodology (especially in the cattle from farms treated at the lower application rates), it is likely that the samples reported as containing no detectable TCDD actually contained TCDD residues, at or below the level of detection. Therefore, some assumptions were made in order to deal with these kinds of results.

Residues were detected in a majority of the samples in the 3 lb/A group. This strongly suggests that the ND samples of this set may have contained residues at, or very close to the limit of detection.

Average residue values were estimated from the results in Table A-4 by averaging the test results for each sample, as follows:

a. Only samples which satisfy the criteria used by the Dioxin Monitoring Program (Table A-7) have been included in the calculations.

b. Data which were reported as non-detected (ND) in the 3 lb/A group have been assigned residue "values" equal to the limit of detection (see discussion above).

c. Samples which were reported as having no detectable residues in the lower application-rate groups have been assigned values equal to the ratio of the application rate in that group to the 3 lb/A rate, multiplied by the specified level of detection for that sample*.

d. When a sample had both positive and ND results, all values were averaged according to assumptions a. through c., above.

e. Due to the limited number (2) of samples from the 4 lb/A group these data were not included in the dietary exposure estimate.

For the purposes of estimating dietary exposure to residues of TCDD in contaminated beef, it seems reasonable to assign real values to samples reported as ND, rather than considering them as zero. The justification for this choice is based on the following reasoning:

A group of highly qualified dioxin analytical chemists established higher critical standards for TCDD analysis than those applied to many routine chemical analyses. It is therefore quite possible that some of the samples which were classified as ND, might actually have been reported as positive were less stringent criteria used.

Further, seven of eleven samples from the 3 lb/A group showed detectable TCDD residues by the established criteria. This suggests that the three samples which had no detectable residues did, in fact, have residues which were close to, but slightly below, the limit of detection. Thus it

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* Non-detected (ND) samples are reported as if the TCDD residues were actually measured (see appendix, Table 3 for modified data) according to the following scheme:
a. 4 lb/A (not included in this evaluation due to small sample size)
b. 3 lb/A treatment group, at the limit of detection reported.
c. 2 lb/A treatment group, at 2/3 the limit of detection reported.
d. 1 lb/A treatment group, at 1/3 the limit of detection reported.
e. 1/2 lb/A treatment group, at 1/6 the limit of detection reported.

seems reasonable to assign values equal to the limit of detection to the "non-detected" samples in this group.

Using the average residue values (which include the assigned positive values for "ND" test results) we find a strong correlation between the rate of applied 2,4,5-T (dosage) and the TCDD residues found in the beef fat. These data are summarized in Table 6. A similar correlation has been observed by Jensen, et al.(ref.10) in a study where cattle were fed forage which had been contaminated with various amounts of 2,4,5-T (containing unspecified, but presumably the same, concentration of TCDD). The observed level of TCDD residues in the adipose tissue appeared to be directly proportional to the added 2,4,5-T in the daily diet. Based on Jensen's observations, it seems reasonable to expect that the level of TCDD in adipose tissues resulting from ingestion of forage contaminated with 2,4,5-T or silvex (and consequently TCDD) would be directly proportional to the rate of application of 2,4,5-T or silvex to that forage.

Therefore, it seems reasonable to assign residue values (to samples which did not have detectable TCDD residues) in some proportion to the amount of 2,4,5-T or silvex used on the forage fed to the cattle. The sensitivity of the method for each particular sample must also be taken into account. Since about 70% of the samples from the 3 lb/A rate showed measurable residues, all ND samples were reported as positive at the level of sensitivity. Samples from fields treated at lower rates were scaled down proportionally (see footnote on page 22).

Finally, Young (ref.32), Zweig (ref.33), and others have observed that the development of increasingly sensitive methods of analysis have permitted detection of residues at continually lower levels, where few

residues had been detected by earlier techniques. It may thus be improper to simply assign a value of "zero" to those non-detected residues, since such an assumption would tend to underestimate residues which might be present, but undetected. The phase one data shown in Table A-4, therefore, have been adjusted accordingly (Table A-6) and averaged and are summarized in Table 6.

Table 6

Summary of Adjusted TCDD Residues in Phase One Adipose Tissue

Application Rate (lb/A)	Range of Residues* (ppt TCDD)	Overall Mean** (ppt TCDD)
3	6.0-46.5	17.1
2	2.0-37.7	10.9
1	2.0-10.0	4.2
1/2	0.3- 1.7	1.2

* Values include adjusted ND samples and are averaged for each animal.
** See Table A-6.

The adipose samples of the phase two 2 lb/A group contained sufficient positive results to compare them with those of phase one. When the phase two data are handled in the same way as those in phase one, the computed mean of 8.63 ppt TCDD compares well with the result in the same group from phase one (10.9 ppt TCDD)

POSSIBLE MECHANISM FOR TCDD INGESTION

A possible mechanism of ingestion of TCDD from 2,4,5-T- or silvex-treated range land or pastures may be suggested by a review of several studies. Mayland et.al. (ref.18), measured the amount of soil ingested by cattle grazing on rangeland. He found that cattle typically ingested up to one kilogram of soil per day, possibly from cropping vegetation close to the soil surface. Under a variety of conditions, TCDD persists at

measurable levels for long periods of time (half-life of 1 year or longer), at or near the soil surface, as shown by Young (ref.32), and Kearney (ref.11) and others. These observations suggest that roots (subthatch) and upper layers of soil in range land and pastures treated with either 2,4,5-T or silvex may constitute a significant reservoir for the TCDD consumed by grazing animals. Thus TCDD residues, either in soil or on vegetation, may account for residues observed to occur in beef animals grazing on 2,4,5-T -treated range land and pasture.

DIETARY INTAKE OF CONTAMINATED BEEF

The reported usage of 2,4,5-T on range land and pasture (ref.2) varies between 1/4 and 2 lb/A, depending on the area of the country, the target vegetation, and other parameters. Rangeland uses of 2,4,5-T are summarized in Table 7. In phase one of the beef study, application of 2,4,5-T on some of the farms studied exceeded these rates (up to 4 lb/A). This raises the possibility that some grazing land is treated at levels considerably higher than the levels reported in Reference 2.

Table 7

Summary of 2,4,5-T-Treated Rangeland*

Method of Application	Target Vegetation	Application Rate (lb/A)	Acres Treated Per Year
Aerial	Mesquite/shinnery oak	1	137,000
Aerial	Mesquite/shinnery oak	1/2	500,000
Aerial	Mesquite/shinnery oak	1/4	400,000
Aerial	Oak Savannah	2	541,000
Ground	Mesquite	1/2	75,000
Ground	Oak Savannah	2	60,000
	Total Rangeland Treated Annually		1,713,000

* Data from Tables 17 and 18, reference 2.

Using the data from Table 7, the weighted mean application rate was calculated and found to be 1 lb/A. This represents an "average" use

of 2,4,5-T on range land. From Table 6, it is seen that a typical TCDD residue level of about 4.2 ppt in contaminated beef adipose tissues is estimated to result from a 1-lb/A treatment of range land with 2,4,5-T or silvex.

DIETARY ESTIMATE

The initial dietary estimate will deal with a group of persons whose dietary beef intake consists solely of contaminated beef. The maximum number of persons in this group can be estimated from the data provided by Lee (ref.17), as follows:

a. Total U.S. Beef Production in 1979 was 21.5 billion lbs.

b. Total U.S. population in 1979 was approximately 220 million people.

c. Therefore, per capita beef consumption in 1979 would be about...

$$\frac{21{,}500{,}000{,}000 \text{ lbs/year}}{220{,}000{,}000 \text{ persons}} = 100 \text{ lbs/person/year}^{*}.$$

d. Total beef produced on range and aerially-treated pastures treated with 2,4,5-T and silvex in any year has been estimated to be from 80 to 137 million pounds dressed weight.**

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* Clearly this "average" figure does not take into account persons who consume no beef whatever, or persons who normally eat larger-than-average amounts of beef.

** Of the total contaminated beef produced on range and aerially-treated pastureland, the majority of the beef, by far, comes from rangeland. Specifically, total beef produced on range areas treated with 2,4,5-T and silvex in one year is estimated to be 73 to 120 million pounds (ref.17). Total beef produced on pasture aerially treated with 2,4,5-T or silvex in a year is estimated to be from 7 to 17 million pounds. Approximately 900,000 acres of pasture receive some 2,4,5-T or silvex treatment from ground application. Since these acres are expected to receive primarily spot treatments, no estimates could be made of the amount of beef which would be contaminated by grazing on these areas. Thus, contaminated beef from ground-treated pasture land was not factored into the exposure analysis. However the amount of such beef is expected to be a minor portion of all contaminated beef coming from treated grazing lands.
Since approximately 9 times as much grazing land is treated with 2,4,5-T as with silvex, it is estimated that about 90% of the beef contaminated with TCDD comes from 2,4,5-T treated grazing land.

e. The percentage of home slaughter beef is estimated to be about 0.9%.

f. Therefore, total beef consumed from home slaughter, raised on treated land is...

80-137 million lbs. x 0.009 = 720,000 to 1,230,000 lbs.

g. Since about 720,000 to 1,230,000 pounds of contaminated beef could be consumed at an average rate of 100 lbs/person/year, it is estimated that between 7,200 and 12,300 persons might consume only contaminated beef (containing 4.2 ppt TCDD in the adipose tissues).

Beef, consumed at 100 lbs/person/year is equivalent to 124 grams/person/day* (approximately 1/3 pound). Assuming beef to contain about 15% (Ref. 18b) fat, a typical daily intake would be about 19 grams of contaminated fat. Based on 4.2 ppt of TCDD residues in beef adipose tissue resulting from the application of 1-lb/A 2,4,5-T to rangeland, an average intake of 80 pg TCDD/person/day would be predicted, assuming all beef to be contaminated. This number represents the dietary intake by a population whose total beef intake was contaminated (home slaughter). Exposure to local populations would be expected to be proportionally higher, if higher rates of application were used (labels permit treatment up to 4 lb/acre).

The average intake of TCDD by local populations consuming TCDD-contaminated beef would be expected to be about 80 pg/person/day during the first year following application of 2,4,5-T or silvex to grazing lands at 1 lb/A. Reference 2 reports retreatment no more frequently than once every 5 years. Since it is known that TCDD declines in soil with a half-life of at least one year (Ref. 11, 32) cattle could reasonably

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* Based on data provided by Schmitt (ref.23), dietary intake of beef, liver and veal would be about 112 grams/day, which agrees well with Lee's data (ref.17), which is based on more recent information.

be expected to ingest decreasing quantities of TCDD from one herbicide application to the next.

It seems reasonable to assume that residues of TCDD in adipose tissues will be directly proportional to the amount ingested. Thus, the decline of tissue TCDD-levels could roughly parallel the decline of soil residues.

If we assume a "typical" half-life of 1 year for TCDD in soil, the average intake of TCDD during each of the 5 years between applications may be estimated (Table 7a).

Table 7a

Estimated TCDD Intake
Intra Application Period *

Year	TCDD Intake (PG/Person/Day)
1st	80
2nd	40
3rd	20
4th	10
5th	5
6th	80**

* Assumes on application of 2,4,5-T or silvex at 1 lb/A, at a frequency of every five years.

** Reapplication.

It should be emphasized that some farmers may choose to treat especially stubborn (herbicide-resistant) weeds in successive years, or at rates higher than 1 lb/A. Herbicide use in the state of Missouri in the beef (phase one) study, for example, was 3 lb/A, with reapplication in two successive years. Thus, some local populations may become exposed to TCDD levels significantly higher than those estimated earlier for "average" 1 lb/A applications at "5 year" intervals.

The following is an estimate of the dietary intake by the U.S. population at large of TCDD from contaminated beef. As shown under "d" above, the estimated volume of beef from animals grazing on 2,4,5-T or silvex-treated areas ranges from 80 to 137 million pounds dressed weight. The total U.S. production of beef is estimated to be 21.4 billion pounds. Thus, the total amount of contaminated beef produced in any one year is estimated to range from 0.4 to 0.6% of the total U.S. beef production*. The dietary exposure of the general population to TCDD from contaminated beef, therefore, is estimated to range from 0.3 to 0.5 pg TCDD/day.

It should be noted that only a very small percentage of grazing land is treated annually with 2,4,5-T or silvex. If the use of these herbicides were to increase, residues in grazing cattle might reasonably be expected to increase proportionately.**

INTAKE OF TCDD FROM CONTAMINATED MILK

We have no information on whether or not it is valid to estimate possible residues of TCDD in the milk of dairy cattle, extrapolated from the TCDD residues in the adipose tissues of beef cattle. It is unclear whether

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* These estimates are based on the amount of beef cattle produced on grazing land treated with 2,4,5-T or silvex during one calendar year. However, if the assumption that cattle acquire TCDD residues by ingestion of contaminated soil is correct, then the real possibility exists that cattle could continually ingest quantities of TCDD over many years. Thus, the total amount of contaminated beef produced annually might be considerably higher than these figures. This is especially true in light of the very long half life of TCDD in soil and low soil mobility which would tend to ensure continued dosing of grazing cattle for a number of years following herbicide application.

** If 2,4,5-T or silvex were to be used on all grazing land, to the maximum extent permitted by the label, (which is highly unlikely) intake of TCDD could be expected to increase to 60 - 100 pg/day (200 x 0.3 to 200 x 0.5 pg TCDD/day).

differences in agricultural practices on pasture (as opposed to rangeland), such as the presuspension label-directed post-application animal reentry interval (for some silvex products and 6 weeks for 2,4,5-T) would lead to significantly reduced ingestion of TCDD by the grazing cattle.

As noted earlier in the discussion of possible mechanisms of ingestion of TCDD from 2,4,5-T- or silvex-treated range land or pastures, TCDD seems to persist in the root area (subthatch) and/or upper layers of soil for long periods of time. The persistence would be far in excess of the post-application intervals mandated by the labels. Therefore, it seems reasonable to assume that dairy cattle, feeding on 2,4,5-T- or silvex-treated pasture, might accumulate residues of TCDD in their tissues very nearly as high as those in beef cattle grazing on similarly treated range land.

We have no information on which to base an estimate of the number of persons in the general population which might consume TCDD-contaminated dairy products. We can only speculate about the level of dietary intake, giving two hypothetical cases.

a. A person whose dietary intake of dairy products consists solely of contaminated dairy products from farms on which cows consume vegetation treated with either 2,4,5-T or silvex, at 1-lb/A.

b. The general population, if all rangeland and pasture were to be treated with either 2,4,5-T or silvex (to the maximum extent permitted by the label) at 1-lb/A .

Assuming that dairy cows have residues comparable to those in beef cattle, and assuming milk to contain about 4% fat, then milk from contaminated

cattle could be expected to contain about 0.17 ppt TCDD*. If the typical dietary intake of dairy products** consists entirely of TCDD-contaminated milk and milk products (containing about 43 grams of fat), then the level of TCDD would then be 190 pg TCDD/day from these dairy products. Exposure to local populations would be expected to be proportionally higher, if higher application rates were used***.

DISCUSSION AND CONCLUSIONS

Assuming recent usage patterns for 2,4,5-T and silvex, the general population would be expected to consume approximately 0.5 pg TCDD/day from contaminated beef. Local populations (i.e. home slaughterers) whose dietary consumption of beef consists of only contaminated beef are estimated to consume 80 pg TCDD/day, on the average. Although difficult to identify, there may be local populations whose dietary consumption of milk and dairy products consists only of contaminated milk and dairy products. This group is estimated to consume up to about 200 pg TCDD/day. There might, theoretically, be local populations consuming only contaminated beef and only contaminated milk and dairy products. They are estimated to consume about 300 pg TCDD/day. Levels of 300 pg TCDD/day might be reached for the general population if all range land and/or all pastures were treated with 2,4,5-T or silvex. However, this scenario is highly unlikely.

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* 4.2 ppt TCDD (Table A) x 0.04 = 0.17 ppt TCDD

** Schmitt (ref.23) estimates the daily intake of Milk and Dairy Products to be about 550 grams, equivalent to about 43 gm of fat. See Table 5-A for computation.

*** The label permits application of 2,4,5-T at rates up to 4 lb./A.

Some assumptions have been made in this analysis which might tend to raise or lower the dietary exposure to dioxin from consumption of TCDD-contaminated beef. For example, in the survey of TCDD residues in beef fat (the phase one study) the levels of TCDD were found to correlate well with the rates at which 2,4,5-T was applied to the grazing land. In order to arrive at this correlation, it was necessary to make the assumption that the animals had consumed only contaminated feed. If, however, this assumption is incorrect, and the animals had grazed on both treated and untreated land, then the dietary intake of TCDD would be expected to be even higher than we have estimated.

The evaluation of TCDD exposure, resulting from ingestion of contaminated beef and dairy products, did not take into account two other factors, neither of which would be expected to affect the exposure estimates by an order of magnitude. These factors could conceivably cancel out one another.

The first factor is the persistence of TCDD in soil, from one year to the next (with a half-life of one year or more). The exposure estimates for the general population were based on the amount of beef produced on range and pasture treated with herbicide within a single calendar year. Since it has been shown that cattle may ingest considerable amounts of soil and/or subthatch material, and that TCDD has a half-life in soil of one year or longer, it is possible that cattle could continue to ingest quantities of TCDD many years after treatment of range land or pasture vegetation with 2,4,5-T or silvex. Thus, the total amount of beef, milk and dairy products which may be contaminated with TCDD could conceivably exceed the levels estimated in this analysis. By not taking this possibility

into account, one would tend to underestimate the exposure to the general population.

Another factor which should be noted is the common practice* of fattening calves and yearlings in feeding lots prior to slaughter. Ingestion of presumably uncontaminated forage and/or grain might tend to dilute residues of TCDD in the adipose tissues. The exact pharmacokinetic mechanisms which apply here are unknown. Since none of the animals in this study were sent to feed lots, their residues were not diluted by this subsequent feeding. Not taking this factor into account would tend to overestimate the exposure.

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* We are aware of the fact that a significant number of beef cattle avoid the feedlots and are sent directly to slaughter. Therefore, dioxin in the meat of these animals would not become diluted by addition of non-contaminated fat. An example of this practice is a local product, Giant Lean. We do not have any data on hand indicating the percentage of beef cattle which are in this category.

DIETARY EXPOSURE TO 2,4,5-T AND SILVEX

This section of the document represents the analysis of the possible intake of 2,4,5-T and silvex by the general U.S. population from residues of 2,4,5-T and silvex in food. In estimating dietary exposure to silvex in treated crops, a range of exposures has been provided, where appropriate. Actual residue data, if available, have been used to estimate realistic dietary exposure levels. Since silvex residues may legally occur up to the tolerance (or interim tolerance) level* in certain foods, it has also been assumed (for purposes of making a conservative estimate), that silvex may be present at this higher level. Where adequate residue data are lacking, exposure estimates were made on the assumption that residues might be present at tolerance levels, but no range of possible residue levels could be given.

The only food crop on which 2,4,5-T is used directly is rice. Since no tolerance for 2,4,5-T residues on rice has been established, the tolerance level could not be utilized in estimating exposure. As explained below, an estimate of 2,4,5-T residues in rice has been based on extrapolation from silvex data.

Ranges of exposure of the general population to 2,4,5-T and silvex residues in food are estimated by taking into account the percentage of each food crop sprayed annually with these herbicides in recent years and the food factor (Ref. 23) which quantifies the percentage of the daily diet repre-

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* The food tolerance represents the upper legally permissible residue level of a pesticide and/or metabolites remaining in or on the crop at harvest time. The only final tolerance for silvex is the one established on pears (post-harvest) at 0.05 ppm (40 CFR 180.340). There are interim tolerances for silvex of 0.1 ppm in sugar cane, plums (prunes), apples, and rice (40 CFR 180.319). There are no food tolerances for 2,4,5-T.

sented by this particular item of food. The food factor is based on the average food intake of 1.5 kg per day by an 18-year old U.S. male. If the percentage of food crops sprayed were to increase, the exposure of the general population to 2,4,5-T and silvex residues in these crops would increase proportionately. For purposes of setting an upper limit, estimates of potential exposure have also been made for the hypothetical situation in which 2,4,5-T and silvex are used to the permissible maximum acreage on food crop, consistent with the pesticide labeling. Although it seems unlikely that 2,4,5-T and silvex would be used to the maximum extent permissible, unforeseeable factors could markedly change current usage patterns so that at least an intermediate exposure might occur.

Exposure to residues of silvex and 2,4,5-T in secondary sources (meat, milk, and eggs) may occur as a result of livestock feeding on treated grasslands and rice by-products such as hay, straw, and hulls and poultry feeding on rice by-products. In addition, exposure to silvex and 2,4,5-T residues in fish may occur as a result of run-off from rice fields treated with these herbicides. A quantitative estimate of exposure to 2,4,5-T and silvex residues in milk and other dairy products has been made for special situations. Although a quantitative evaluation of the exposure to silvex and 2,4,5-T residues via other secondary sources cannot be made at this time, a qualitative discussion follows in a later section.

SILVEX RESIDUES IN THE HUMAN DIET

The results of the dietary analysis for silvex are given in Tables 8 and 9. Table 8 gives a range for the dietary intake by the general population estimated from residues actually found on the treated crops (where known),

and from tolerance levels. The percent commodity annually treated (pre-suspension rate) and the food factors were used in calculating total dietary burden.

TABLE 8

Estimated Dietary Exposure of Silvex

Crop	Possible Residues (ppb)	Percent Crop treated	Food Factor (%)	Rate of Ingestion[3] (ug/day)	Dietary Exposure[4] (ng/kg bw/day)
Rice	12[5] - 100[6]	0.10[1,7]	0.55[2]	0.001-0.008	0.004 - 0.011
Sugar	100[6]	2.6-4.6	3.64[2]	0.141-0.251	2.028 - 3.588
Plums	100[6]	5.4[1]	0.13[2]	0.011	0.150
Apples	42[9]- 100[6]	11.0[1]	2.54[2]	0.176-0.419	2.515 - 5.987
				Total:	4.7 - 9.7 ng/kg/day

1 Ref.17.

2 Ref.23.

3 Based on 1.5kg - daily diet

4 Based on average weight of 70kg per individual

5 In reference 7, 21 samples were at or below 10 ppb, the limit of detection. The Agency made the conservative estimate that residues could be present up to 10 ppb, and therefore, substituted 10 ppb for these 21 samples which in turn were averaged with the 6 positive estimates of 10, 20, 10, 30 and 30 pb. The average (n=27) is 11.85± 0.36 ppb; the average was rounded off to 12 ppb.

6 40 CFR 180.319.

7 There are approximately 3000 acres of rice treated with silvex in a total of 2,979,000 acres (ref. 7). The % of crop treated is: 3000/2,979,000 x 100 = 0.10%

8 Ref.34; 15.3 to 28.6% domestically grown sugar cane is treated with silvex; 2.6-4.6% domestically consumed sugar has been treated; sugar beets are not treated with silvex.

9 See discussion in text on p. 36.

Table 9 provides a range for the dietary intake by the general population in the hypothetical situation of maximal treatment of the crops consistent with the labeling. This situation, although highly unlikely, gives an estimated maximum level of dietary exposure from presently registered uses of silvex.

TABLE 9

MAXIMUM ESTIMATED DIETARY EXPOSURE TO SILVEX

Crop	Possible[1] Residues (ppb)	Percent[2] Crop Treated	Food[1] Factor (%)	Rate of Ingestion (ug/day)	Dietary Exposure (ng/kg BW/day)
Rice	12-100	100	0.55	0.10-0.82	1.42-11.71
Sugar	100	24[3]	3.64	1.31	18.72
Plums	100	12	0.13	0.023	0.334
Apples	42-100	100	2.54	1.60-3.81	22.86-54.43
				Total:	43.3-85.2 ng/kg BW/day

1 Data from Table 8.
2. Figures represent maximum acreage treatable consistent with the labeling. Estimates for sugar and plums utilized information provided in Ref. 17.
3. U.S. Production of cane sugar (1977-1979) = 2.6 million short tons. Total sugar consumption = 11 million short tons, cane and beet sugar, Ref. 34

The maximum treatable crops are 100% of all U.S. grown rice, sugar cane, and apples, but only 12% of plums (including prunes), and 10% of pears. Of all plums (including prunes) only Italian prunes are listed on the pesticide label treatment with silvex, representing 12% of all plums grown in the U.S. Silvex may be used only on Anjou pears, corresponding to 10% of all pears grown in the U.S. The dietary exposure estimates shown in Table 9 might also represent the levels of exposure under recent use practices for certain local populations which could conceivably consume exclusively contaminated foods of each of the four types considered.

DIETARY EXPOSURE FROM RICE

Residues of silvex have been detected in rice treated with silvex grown in Arkansas, Louisiana, and Texas at levels ranging from 10 to 30 ppb, with an average of 12 ppb (Ref. 7). The calculations are fully explained in footnote 5 of Table 8. As seen in Tables 8 and 9, the possible silvex residues are expressed as a range from 12 (avg) to 100 ppb, the latter value being the interim tolerance (0.1 ppm).

Certain ethnic groups eat more rice daily than the general population. Consideration must, therefore, be given to possibly higher dietary intake of silvex from treated rice by these ethnic groups. If we assume that these groups (whose numbers cannot be easily estimated, but which could be quite large) substitute rice for potatoes in their daily diet, the food factor could increase from 0.55% (rice food factor) to 5.5% (potato food factor) which represents a ten-fold increase in the potential dietary intake of silvex from rice.

DIETARY EXPOSURE FROM SUGAR CANE

The use of silvex is recommended for sugar cane but not sugar beets. Zygadlo (Ref.34) estimated that 2.6-4.6% of all domestically consumed sugar has been treated with silvex. Based on our review of current EPA files, it does not appear that silvex residues on sugar have been analyzed. Therefore, it is assumed that residues may be present at the maximum permissible level, i.e. the interim tolerance of 0.1 ppm (100 ppb). The percentage of the crop treated (Table 8) represents the percentage of sugar annually produced in the U.S. in recent years which is treated and consumed. The corresponding value in Table 9 is the percentage of U.S. treated sugar consumed if 100% of the U.S. sugar cane were to be treated with silvex.

DIETARY EXPOSURE FROM PLUMS

Table 9 reflects the fact that only some plums (Italian prunes) are treated with silvex, accounting for the fact that the maximum treatable crop is only 12% (the percent of total U.S. plum production consisting of Italian plums). Based on our review of current EPA files it does not appear that analyses of silvex residues on plums or prunes have been performed. We, therefore, assume that residues may be present at the interim tolerance of 0.1 ppm.

DIETARY INTAKE FROM PEARS

Silvex is applied to Anjou pears trees after harvest. Therefore, any residues of silvex appear in the following years crop. The Agency has no record of silvex analyses on pears. Based on the post-harvest use pattern, we do not believe that a strong possibility exists for silvex residues to occur in pears and have, therefore, excluded pears from the dietary exposure estimate.

DIETARY EXPOSURE FROM APPLES

We are aware of a study dealing with treatment of apples with silvex (Ref. 6) In this study, McIntosh apples were treated on the tree with a 20 ppm solution of silvex (according to label instructions) and were analyzed for silvex residues at different daily intervals up to harvest time, after 2 weeks storage, and 4 months' storage (Ref.6). The following results were obtained.

	Silvex Residues* At Harvest	Silvex Residues After Storage for... 2 weeks	4 months
Unwashed apples	32 ppb	42 ppb	35 ppb
Washed apples	27 ppb	26 ppb	16 ppb

* 14 days after last application

We have taken the range between the average residue (42 ppb) found in unwashed apples (stored for two weeks) and the interim tolerance, 100 ppb, to estimate the daily dietary exposure of the general population. If one wishes to make other assumptions (e.g., washed apples), appropriate exposures can be estimated by consulting the original paper (Ref. 6).

DIETARY EXPOSURE FROM SECONDARY (INDIRECT) SOURCES

A possibility exists that the general U.S. population may ingest silvex residues from meat, milk, poultry and eggs, from livestock and chickens ingesting feed contaminated with silvex. Such contaminated feeds may originate from the use of silvex on rangeland, pasture, and rice. There are a number of studies showing the decline of 2,4,5-T residues in grass after treatment. No comparable studies have been found for silvex. For example, Baur, et al. (ref.3) applied 2 lb/A of the 2-ethylhexyl ester of 2,4,5-T on a plot containing live oak and grasses and observed that 2,4,5-T acid and ester declined as follows:

Time After Treatment	Acid	Ester	Acid + Ester (a.e.)[1/]
1 month	4.06 ppm	2.89 ppm	5.96 ppm
6 months	0.06 ppm	0.17 ppm	0.17 ppm

In another study, Bovey and Baur (ref.5) applied 0.5 and 1.0 lb/A of the propylene glycol butyl ether ester of 2,4,5-T on grasslands in Texas and observed the following decline of 2,4,5-T residues.

Time After Treatment	ppm 2,4,5-T*
Initial Residues	65.1
6 weeks (1.5 months)	1.6
26 weeks (6 months)	0.013

* average of 5 locations.

1/ Acid equivalent

In order to translate these data to possible silvex residues in milk from cows grazing on treated pastures, a study by Bjerke, et al. (Ref. 4) proved helpful.

Bjerke, et al. (Ref. 4) showed that feeding milk cows 1000 ppm of silvex in their daily feed resulted in an average of 100 ppb residues of silvex in the milk at steady state.

If we assume, therefore, that the environmental fate of silvex and 2,4,5-T are similar, we can use the data of Bovey and Baur (Ref. 5) to estimate (by interpolation) the amount of 2,4,5-T, and, therefore, silvex residues, which would remain on treated grass 1 week after the last application (There is a 1 week restriction of dairy animals entering silvex-treated pastures). This value of 50 ppm of silvex in feed, is equivalent to about 5 ppb (0.005 ppm) of silvex residues in milk, based on an extrapolation of experimental data (Ref. 4). This extrapolated value is below the sensitivity of the method (0.05 ppm). The average male ingests about 500 g of milk and dairy products (ref.23) per day, expressed as of fluid milk. At 5 ppb in the milk, therefore, a person consuming only milk from dairy animals grazing on pastures recently treated with silvex would ingest 2.5 ug of silvex daily.

2,4,5-T DIETARY EXPOSURE

There are potentially two major sources of dietary intake of 2,4,5-T from food:

1) the direct application of 2,4,5-T to rice
2) indirect exposure from meat, milk, poultry, and eggs derived from chicken and livestock fed on contaminated feed.

Beef and dairy cattle may graze on rangeland and pasture that has been treated with 2,4,5-T. This possibility is exemplified by the obser-

vations f m beef studies phases I and I , discussed in the previous section, which showed that TCDD residues were detected in beef cattle from 2,4,5-T-treated fields. However, TCDD and 2,4,5-T have different metabolic pathways, and no extrapolation from TCDD to 2,4,5-T residue can be attempted without further experimentation. We are not aware of any specific studies of 2,4,5-T residues in rice or meat or milk products, so that our dietary analysis will be done by extrapolation from other data on similar compounds, e.g. silvex.

An alternate method of estimating 2,4,5-T exposure, discussed above under silvex dietary exposure, is suggested by the studies of Bovey and Baur (ref.5) who showed that residues of 1.6 ppm 2,4,5-T remained in grass 6 weeks after treatment with 2,4,5-T. That same period is also the restricted interval for dairy cows reentering areas treated with 2,4,5-T. Based on studies of Bjerke et al. (Ref. 4), residues of 1.6 ppm in the feed are estimated by extrapolation to yield residues that are below 1 ppb in the milk. Based on this calculated value of < 1 ppb, we estimate that the daily exposure to those individuals whose 500g/day-fluid milk might be solely derived from contaminated milk, is less than 0.5 ug of 2,4,5-T per day.

From the work of Devine (Ref.7), it was shown that silvex residues from silvex-treatment of rice actually do occur, and are at an average level of 12 ppb. Since the use patterns for 2,4,5-T and silvex are quite similar, and since the two compounds are closely related chemically, and since they both have similar environmental characteristics (such as

similar half-lives), we may estimate the following dietary exposure to 2,4,5-T for the general population from the silvex data on contaminated rice:

Possible residue: 12 ppb

Percent crop annually treated: 10.9% (Ref.17)

Food Factor: 0.55(Ref.23)

Estimated Rate of Ingestion: 0.011 ug/day/person

Therefore, the estimated dietary exposure, based on recent usage patterns would be 0.154 ng/kg/day, based on 70 kg body weight.

If the hypothetical, but highly unlikely, situation case may be considered, in which all rice is treated with 2,4,5-T, the dietary exposure of the general population would increase to 1.40 ng/kg/day. This might also represent the exposure for certain limited populations which might eat contaminated rice exclusively.

We might also consider the possibility that certain ethnic groups could eat up to 10 times as much rice as the general population and might, therefore, be exposed to between 1.5 and 14 ng/kg/day, a ten-fold increase in exposure.

DIETARY EXPOSURE TO TCDD FROM DEER AND ELK

The EPA Deer and Elk study was begun on/or about October 22, 1977 (Ref.28) with the collection of perirenal fat samples from animals in both Washington State and Oregon. The rationale for this study was that, since extensive use of 2,4,5-T on forests (reportedly on more than 250,000 acres in Oregon alone) was occurring in these states, the possibility of contaminated game needed to be evaluated. Animals were, therefore, sampled from forested areas of these two states where 2,4,5-T was currently in use. Table 10 summarizes the sampling program.

Table 10

Summary of Deer and Elk Sampling

Collection Dates	Number of Samples (M/F)		Description
10/22/77	9	(7/2) Deer	Taken between Aberdeen, Washington
10/23/77			and the Pacific coast
10/31/77 11/21/77	12	(3/9) Elk	Olympic Peninsula of Washington on several trips.
11/05/77	10	(7/3) Deer	Tillamook area of Oregon from hunters' catches.
12/03/77	15	(0/15) Elk	Single herd between Coos Bay and Roseburg, Oregon.

The program in the Northwest was coordinated by Michael Watson, a toxicologist with EPA's Region X office. Dr. Watson enlisted the assistance of Mr. Reade Brown (Chief, Game Management, Washington Department of Game, Olympia, Washington) and Mr. Jerry MacLeod (Biologist, Oregon Department of Fish and Wildlife, Portland, Oregon) who supervised the sample collection and quality assurance (Refs.29,30)

Dr. Watson provided the appropriate sampling protocol to be used; in addition, he supplied all necessary equipment (which had been rigorously cleaned in the laboratory to avoid precontamination with dioxins), so that the deer and elk adipose tissues could be reliably sampled. Complete capture records were required for each sample.

Following their collection, the adipose tissue samples were frozen within 24 hours, shipped to Dr. Watson under refrigeration and held in deep freeze for approximately one year (until 11/14/78). At that time they were shipped to the EPA Toxicant Analysis Center, in Bay St. Louis,

Mississippi, for cleanup, extraction and encoding. Extracts, including appropriate blanks and quality control checks were subsequently forwarded (on 9/06/79) to both the EPA laboratory at Research Triangle Park, North Carolina and to Wright State University, Dayton, Ohio.

RESULTS AND DISCUSSION

Table 11 summarizes the deer and elk data. The results of the analyses of the Washington deer fat were inconclusive. Four of 5 results require reanalysis of the samples because of low recoveries, and one result was ND at a limit of detection of 2 ppt. An additional sample was not run at all, due to small amount of adipose taken.

The results of the analyses of the Oregon deer fat samples averaged 10.5, 12 and ND(4)ppt. Assuming residues to be present at the limit of detection for the ND data*, the mean residues for this set would be about 8.8 ppt. Two of the six samples require reanalysis due to low recoveries.

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* The rationale for assuming residues to be present at the limit of detection in samples with reported ND data, while not as defensible as in the case of the beef data, nevertheless seems reasonable for the following reasons:

(1) There were a number of confirmed positive results from deer and elk taken from the same area of the respective states.

(2) We have no evidence which would lead us to conclude that the ND results reflect no dioxin at all in these samples.

(3) We believe it to be prudent to resolve uncertainties in data on the side of public safety.

Table 11

Summary of Deer and Elk Data[a]

Animal	TAC #	Reported[b] TCDD - ppt RTP	WSU	Animal	TAC #	Reported[b] TCDD - ppt RTP	WSU
deer	WA-D-1	ND(2)[c]	ND[d]	deer	OR-D-1	ND(4)	ND[d]
deer	WA-D-4	ND[d]	NA	deer	OR-D-5	12	31[d]
deer	WA-D-8	7[d]	ND[d]	deer	OR-D-6	7	14
elk	WA-E-2	9	NA	elk	OR-E-7	24	29
elk	WA-E-4	21	21	elk	OR-E-8	4	ND(10)
elk	WA-E-5	12	ND[d]	elk	OR-E-9	5	ND(8)
elk	WA-E-7	ND[d]	ND[d]	elk	OR-E-11	ND(2)	ND(8)
elk	WA-E-8	54	68				

ND Not Detected(see Table A-7 for DIP Criteria)
NA Not Analyzed due to limited amount of sample.
a. Ref.1.
b. Corrected for recovery losses
c. Parenthetic values are limits of detection for the analysis.
d. Recoveries below 50%. Samples to be rerun.

TAC = Toxicant Analysis Center (EPA Lab. in Bay St. Louis, MS)
RTP = EPA Lab at Research Triangle Park, N.C.
WSU = Wright State University, Dayton, OH

The results of the analyses of the Washington elk indicated much higher residues of TCDD in the fat, with average values of 9, 12, 21 and 61 ppt. The simple mean for this group of samples was 26 ppt. Of the ten results, three samples require reanalysis due to low recoveries, and one sample was not run due to limited size. The high values were confirmed by both analytical laboratories (21 & 21 ppt, and 54 & 68 ppt).

The results of the analyses of the Oregon Elk showed residues of TCDD in 3 of 4 adipose samples, with average values of 5, 7, 7 and 26.5 ppt TCDD. The mean for this group of samples */ would be 21 ppt. Of the

*/ See footnote on page 42.

8 results, four were ND at good limits of detection. The high result was confirmed by both participants (24 & 29 ppt).

DIETARY ESTIMATE

The following assumptions were used in the dietary estimate.

a. For the local populations which might consume the meat of wild game, deer and/or elk meat are substituted partially or wholly for beef in the diet, on an individual basis.
b. The fat content of deer meat may range from 4% - 17%, depending on seasonal variations.*/
c. The fat content in elk meat is essentially the same as that of deer meat.**/

Residues of TCDD ranged from ND(2-13) to 31 ppt in contaminated deer fat, and from ND(0.8-25) to 68 ppt in contaminated elk fat. TCDD intake from contaminated deer or elk is summarized in Table 12. This is a worst case situation in that meat consumed consists of only contaminated deer or elk meat. We do not have sufficient information to estimate the number of persons in this group.

*/ Deer store fat during the spring and summer, for use over the winter. In a study by Medin and Anderson, (Ref. 18a), Colorado Mule Deer were analyzed for fat content and found to contain 5-17% (males) and 7-13% (females), the range reflecting seasonal variation. However, Dr. J. Pennington, a nutrition expert with the US Food and Drug Administration reports (Page 186), that raw venison contains about 4% fat. However, Dr. Pennington reported that consumption of venison was much higher per capita (6-8oz. portions) than typical beef consumption (4 oz). Thus, consumption of deer fat contaminated with TCDD, might be comparable to consumption of beef with twice the fat content for purposes of estimating dietary exposure to TCDD.

**/ We could find no specific information on the amount of fat in elk meat. We have no reason to believe that elk contain more or less fat than deer. Therefore, the assumption that deer and elk meat contain comparable percentages of fat seems reasonable.

Table 12

Dietary Intake of TCDD From Contaminated Deer or Elk

Animal	TCDD in Fat (ppt)	TCDD in Meat(ppt)*	Dietary Intake**/ (pg/person/day)	pg/kg bw/day***/
DEER	ND(2-13) - 31	0.08 - 5.27	9.9 - 650	0.14 - 9.3
ELK	ND(0.8-25)- 68	0.03 - 11.56	3.7 - 1430	0.05 - 20.5

*/ Assumes 4% - 17% fat, depending on season. Computed range is the lowest percentage fat multiplied by lowest limit of detection to the highest percent fat multiplied by the highest detected residues. Thus 2x0.04 = 0.08; 31x0.17 = 5.27; 0.8x0.04 = 0.03; 68x0.17 = 11.56

**/ Assumes deer and elk meat is consumed at the same rate as beef is consumed (124 gms/person/day.).

***/ Assumes a 70 kg person

Thus, a person consuming contaminated deer meat once a month (or for a period of 12 days following the hunting season), for example, could possibly ingest from 1.7 to 111 pg 2,3,7,8-TCDD/kg-BW/ year. Similarly, a person consuming contaminated elk meat could, at that rate, ingest from 0.6 to 246 pg 2,3,7,8-TCDD/kg BW/year.

An informal survey of ten persons was taken during June, 1980 (Ref.9) to determine typical consumption of deer and elk meat. The 10 people contacted resided in Oregon, and reported having deer and/or elk meat on hand. One person consumed venison 4 times a week until all meat on hand was gone; six people consumed venison or elk meat about once a week; the other three persons consumed venison or elk about once every two weeks, until the meat was gone. Typical consumption of this group of people seemed to be about once a week. It is not known whether any other persons were contacted who did not have game on hand, or whether this group of persons were selected because it was suspected that they were likely to have game on hand.

Individuals eating contaminated deer or elk meat once a week (or at a comparable rate) would have a dietary intake of TCDD ranging from 7.3 to 485 pg TCDD/kg BW/year from contaminated deer meat, and from 2.6 to 1070 pg TCDD/kg BW/year from contaminated elk meat, assuming a year's supply of meat was available.

If less meat were available, estimates would be correspondingly lower. Thus, if only a six month's supply were on hand, dietary intake might be one half of the estimated rate.

In addition, if, in fact, individual consumption of game is higher than beef consumption for a particular meal then intake of TCDD from this source may be higher than is reflected in this estimate.

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APPENDIX

ESTIMATION OF EXPOSED POPULATION AND DURATION OF EXPOSURE TO 2,4,5-T

In order to estimate the annual exposure of 2,4,5-T to a population and to assess the resultant risk, the following information must be taken into account:

1. Number of individuals within a defined occupational group.
2. Duration of daily and annual exposure for these specified occupational groups.

These numbers are for the most part not available as hard data for 2,4,5-T and silvex uses, and consequently an estimation technique has been utilized. Annual exposure and the number of applicators for several types of uses have been estimated by considering total acreage treated, area treated/unit time, the timing of application, and by making certain assumptions about duration of exposure*. The basis of data for "total acreage", "area treated/ unit time", "timing of application", and other use information is contained in Reference 2, (hereafter referred to as the Report). The specific uses for which these estimations were done are the following:

- Forestry
  - aerial
  - ground broadcast
  - backpack mist blower
  - backpack spray
- Range and Pasture
  - aerial
  - backpack spray
- Rice
  - aerial
- Rights-of-Way
  - aerial
  - ground
    - selective basal
    - cut stump
    - mixed brush
  - handgun
  - truck broom
  - railroad tracks
  - electric power lines

The results of these estimations are summarized in Table A-1.

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* In some cases we have considered that the hours exposed were equivalent to the hours worked, as is explained in the text.

TABLE A-1

2,4,5-T - Estimation of the Number of the Exposed Population And Duration of Exposure(1)

1	2	3	4	5	6	7	8	9	10
USE PATTERN	EXPOSED GROUP	TOTAL ACREAGE (1000's)	TREATED ACREAGE (A/hr)	TREATMENT DURATION (hrs/day)	RATE (lb.ai/A)	days/yr (avg)	EXPOSED POPULATION (no.)	DAILY EXPOSURE (hrs.)	ANNUAL EXPOSURE (hrs/yr/person)
				FORESTRY					
1. Aerial	Pilots	876	60	2	1.5-3	100	73	2	200
	Mixer/Loaders	876	60	2	1.5-3	100	73-145(2)	8	800
2. Ground Broadcast	Boom Tr. Opr.	140	6.5(avg)	4	2-3	60	90(3)	4	240
	Mixer/Loaders	140	6.5(avg)	4	2-3	60	90-180(3)	8	480
3. Backpack Mist Blower	Applicators	24	0.5	8	2	60	100	8	480
4. Backpack Sprayers	Applicators	125	0.5	8	2	100	300	8	800
				RANGE AND PASTURES					
1. Aerial	Pilots	1,578	200	6	0.5-2	10	130	6	75
	Mixer/Loaders	1,578	200	6	0.5-2	10	130-260(2)	8	100
	Flagpersons	1,578	200	6	0.5-2	3	800 (4)	8	25
2. Backpack Sprayers	Applicators	1,060	0.6	8	0.5-2	10	20,000	8	80
				RICE					
Aerial	Pilots	292	80	2	1	6	307(5)	2	12
	Mixer/Loaders	292	80	2	1	6	307(5)	8	48
	Flagpersons	292	80	0.6	1	<1	6500-9500(5)	0.6	0.6

TABLE A-1 (continued)

2,4,5-T - Estimation of the Number of the Exposed Population And Duration of Exposure(1)

1	2	3	4	5	6	7	8	9	10
USE PATTERN	EXPOSED GROUP	TOTAL ACREAGE (1000's)	TREATED ACREAGE (A/hr)	TREATMENT DURATION (hrs/day)	RATE (lb.ai/A)	days/yr (avg)	EXPOSED POPULATION (no.)	DAILY EXPOSURE (hrs.)	ANNUAL EXPOSURE (hrs/yr/person)
				RIGHTS OF WAY					
1. Aerial	Pilots	206	20(6)	4	8	100	25	4	400
	Mixer/Loaders	206	20(6)	4	8	100	25-50	4	400
2. Ground									
a. Selective Basal	Applicators	235	0.5	6	6.4	170	1380	6	500
b. Cut Stump	Applicators	10	0.5	6	4	170	60	3	500
c. Mixed Brush	Handgun Appl.	30	0.5	6	6	110	356	6	660
	Truck/Boom Appl.	50	1	6	0.8	110	178	6	660
d. Railroad	Crew (of four)	100	10	4	2-6	66	114	4	264
e. Electric Power	Applicators	44	0.5	6	6	110	400	6	660

(1) Reference 2.
(2) Crew consists of 1 pilot + 1-2 mixer/loaders.
(3) Crew consists of 1 tractor operator + 1-2 mixer/loaders.
(4) Assumes that the average local ranch is 4000 acres in size, and employs 2 flagpersons.
(5) These figures are quoted from (1).
(6) The 20 A/hr figure is taken from Table 35, chapter 5 of reference 2, above.

It should be noted that we are more certain about our estimate of the total number of exposure-hours for each specified use and use pattern than we are about the exact number of individuals in each group and the number of hours worked by each individual.

Since for each occupational group...

total # exposure hrs[a] = # of workers[b] x averace # hrs worked or exposed[c]

even if (b) and (c) were in error, they would vary inversely and (a) would not change appreciably.

SPECIFIC EXPLANATIONS OF TABLE A-1

Column 3 - Total Acreage

This number is taken from tables or the text of Part 5 of the Report. For example, the first figure under aerial forest, 876,000 A, is found in Table 12, p. 5-95 of the report.

Columns 4 and 5 - Acreage Treated/Unit Time - Duration of Treatment

These numbers are usually found in the text or in the "Calculation Summary" of the Report. This is an estimated average based on the descriptive portion of the Report or the Calculation Summary Table. For example, on p. 5-92 of the Report it is stated that it may take 10-30 minutes to treat 30 acres by helicopter. As stated in Calculation Summary No. 1, one site of up to 180 acres usually 1-3 hours to treat with herbicide. Based on this specific information we have chosen 60A/hour as the acreage treated per unit time and 2hrs/day as the duration of treatment.

Column 6, Application Rates

Application rates are found in the text of the Report or in Calculation Summary tables. When a range is given (e.g., 1.5-3 lb/A) the approximate

weighted or arithmetic average, e.g. 2 lbs/A, is used for further calculations.

Column 7

The days-per-year figures are an indication of the number of days 2,4,5-T might be applied for a certain use throughout the year. Since most applications are made by professional applicators, it is assumed that the same crew would be applying 2,4,5-T in different areas. According to the information on page 5-90 of the Report, 2,4,5-T is applied during 3 periods of the year: Feb-March; May-June; July-Sept. Assuming inclement weather conditions (rain, wind), we estimate, therefore, that the aerial crew for forestry use will be applying 2,4,5-T for 100 days/year.

Column 8 - Number of Individuals of Exposed Population

This number is calculated as follows:

$$\text{\# Spray crews} = \frac{\text{Total acreage (Col.3)}}{\text{A/hr (Col.4) x hrs/day (Col.5) x days/yr (Col.7)}}$$

For example $$\text{Spray crews} = \frac{876{,}000}{60 \times 2 \times 100} = 73$$

If, according to the Report, a spray crew consists of one pilot and 1 to 2 mixer-loaders, then (as in the example above) there would be 73 pilots and 73-146 mixer-loaders. A risk assessment might be performed on the low and high range of the exposed population. (For one use, aerial application of 2,4,5-T to rice, the number of pilots is fairly well confirmed to be around 300.)

Column 9 - Daily Exposure

We assume pilots to have a short exposure. In most cases only two hours of flight time are permissible during an average work day. Thus, pilots

usually are listed as being exposed for 2 hours/work day. The mixer-loaders in aerial application are engaged in the loading and mixing of pesticides during the actual application period (2 hours) but are assumed to be working on other tasks throughout the workday (6-8 hours) without a change of clothes. Thus, we believe that the workers will be exposed to 2,4,5-T during the entire work day by contact through the skin from wet, pesticide-contaminated, work clothes.

Column 10

Annual Exposure = Days/yr. (Col.7) x daily exposure (Col.9).

SPECIFIC DATA POINTS AND ASSUMPTIONS*

Forestry - Air Application

Total Acreage - 876,500A (Table 12, p. 5-95).

Acreage Treated - 180A/day; usually 1-3 hours (Calc. Summary No.1).

Application Rate - 1.5-3 lbs/A (Calculation Summary No. 1)

Days per year - 100 days (Table 10, p. 5-90)
e.g. Pacific Coast (pine release): Feb-March, May-June and July-Sept

Daily Exposure - As discussed previously, the assumption is made that the pilots are exposed 2 hours/day based on actual flight time and change clothes at the completion of the flight. On the other hand, the mixer-loaders are assumed to remain in the field engaged in other tasks, wearing contaminated apparel during the normal working day of 8 hours. Therefore, exposure is estimated at 2 hrs/day for pilots and 8 hrs/day for mixer-loaders.

Forestry-Ground Broadcast (Tractor-applied)

Total acreage: 140,000A (Table 12, pp. 5-100).

Application Rate: 2-3 lbs/A (Table 14).

Acreage Treated: 5-8 A/hour (p. 5-99).

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* All other data points are found in Table 1.

Forestry-Ground Broadcast (Tractor-applied - Continued)

Duration of Treatment: 4 hours/day (p. 5-99).

Days/year: approximately 60 working days (Mid-April- Mid-July);

Daily Exposure: We are assuming that the mixer loaders work a full work day beyond the 4-hours of application, and are, therefore, exposed to the pesticide during the entire work day of 8 hours.

Forestry - Backpack Mist-Blower

Total Acreage: 24,000A (Table 15, p.5-103)

Rate of Application: 2 lbs/A (p. 5-101)

Days/year: May to July - approximately 3 months or 60 working days

Treated Acreage: 3-5A/day (p. 5-101) or approximately 0.5A/hour

Forestry - Backpack Sprayer:

Total Acreage: 125,000A (Table 16, p. 5-106)

Application Rate: 2 lbs/A (p. 5-104)

Treated Acreage: 3-5A/day, 4 hrs application 8 hrs work day

Day/Year: May-July; Nov-March; approximate 100 days/yr.

Range and Pasture:

Aerial Application

Total Acreage: 1,578,000A (Table 17)

Treated acreage: 100-300A/hr. (p. 5-111) av. 200A/hr.

Application Rate: 0.5 - 2 lbs/A (Calculation Summary No. 2); weighted average = 1.0 lb/A

Duration of treatment: 6 hours/day (p. 5-111) (3 hours AM and 3 hours PM)

Aerial Application (continued)

Days/year: Pilots and Mixer/Loaders: 1-4 wks., 10 days (avg) (p. 5-111)

Flagperson: about 3 days (assumes 4000A farm at 1200A/day

Daily exposure: It is assumed that the pilots change clothes after each flight period, making a total of 6 hours exposure. The other workers are assumed to retain the same work clothes during an 8-hr workday, resulting in 8 hours of exposure.

Exposed Population: Assuming the average ranch to be of 4000A size and 2 flag persons per ranch, it is estimated that (1,600,000: 4000) X 2 = 800 flag persons will be employed. Other populations were estimated by the calculation shown on p. 52.

Range and Pasture

Backpack Sprayer:

Total Acreage: 1,060,000A (excluding mesquite, table 18,

Acreage: 3-5A/day (p. 5-113)

Duration of Treatment: 8hrs/day

Rate: 0.5-2 lbs/A (Table 18); weighted average: 0.6 lb/A

Rice

The best available information is that 97% rice treatment is by air (Report, p. 5-142).

Total acreage: 292,000A (p. 5-144)

Treated Acreage: 46A/35min or approximately 80A/hour (p. 5-148)

Duration of Treatment:
Calculated 2 hours/day and 6 days/year for pilots and loadmen.
Calculated 0.6 hrs/year for flagpersons.

Rice (continued)

Exposed Population: 307 pilots (p. 5-149). For each pilot there is one loadman. Since the number of pilots is known, the yearly exposure hours were calculated by the equation on p. 52. There are roughly 6500 rice farmers with an average of 1.5 flagpersons being supplied by each farmer; thus, the number of flagpersons range from 6500-9500 (p. 5-148).

Rights-of-Way

1. Aerial

Total acreage: 206,256 A (Table 19)

Treated acreage: 6-15 A/hr. (p. 5-120)
20 A/hr. (p. 5-191); this number is used

Typical Work Day: 4 hrs (p. 5-120)

Work Crew: 50-75, consisting of pilot
mechanic/service
mix-truck driver = mixer loader

Duration: 22 weeks or about 100 days

2. Ground

a. Selective Basal (p. 5-136, calculation summary No. 7)

Total Acreage: 235,000 A

Treated Acreage:

0.5 A/hr or 3 A/day
for 6 hour work day

Duration of treatment

34 weeks or approximately 170 days

Nos. of exposed Population

work crew consists of

driver-mixer
2 spray men

$$\text{No of work crews} = \frac{235{,}000}{170 \times 3} = 460$$

Total nos. of persons = 460 x 3 = 1380

b. Cut Stump (calculation Summary 8)

Total Acreage: 9,901 A

Dosage: 3.2 lb/A - 4.6 lb /A
Average: 4 lb /A

Duration of treatment

34.7 weeks or 170 days / year

Application time: 6 hrs / day

Application rate: 0.5 A/hr
(based on estimate)

No. of workers exposed:

$$\frac{10,000}{3 \times 170} = 20 \text{ work crews}$$

Crews made up of 2 spraymen
1 truck driver-mixer

Total = 60 persons

(Summary Table 8 lists 76 exposed personnel; this must include 1 supervisor, who is not included in our estimates. We also assume that all persons are exposed during entire 6 hour work day)

c. Mixed Brush - Handgun (Calculation Summary 9)

Total Acreage: 29,400 A

Treated Acreage: 0.5 A/hr
6 hour day
3 A/ day

Duration of Annual treatment: 110 days

Exposed Population: 89 work crews consisting of 4 persons

Total: 356 persons

(Note: There is an error in Calculation Summary 9; should be 89 work crews instead of 39, as written.)

c. Mixed Brush (Roadside)

Truck - broom applicators
(calculation Summary No. 10)

Total Acreage: 58,447 A.

Duration of treatment:

22 weeks or 110 days

Nos. of exposed workers:

Driver, mixer loader
Sprayman
90 work crews

Treated Acreage: Given 90 work crews (p. 5-139) and 110 days x 6 hrs and 60,000 A to be treated, the calculated treatment rate is approximately 6 A/day or 1 A/hour.

d. Railroad Crew (Calculation Summary 11)

Total Acreage: 100,000 A

Treated Acreage: 10 A/hr

Duration of treatment

4 hrs / day

13 weeks / year

260 hrs / year

Total population

38 crews x 4= 112 persons

e. Electric Power (Calculation Summary 12)

Total acreage: 44,000 A

Treated Acreage: 0.5 A/hr

Duration:

6 hrs / day

110 days / year

Total nos. of persons:

Driver / mixer-loader
2 spraymen

Nos. of crews:

$$\frac{44,000}{330} = 133$$

Total nos. individuals = 400

TABLE A-2

Estimated Occupational Exposure to 2,4,5-T (Refs.14,12)

WORKER NO.	WORKER ACTIVITY	WORKER WEIGHT (Kg)	MIN. TIME EXPOSED A*	MIN. TIME EXPOSED B*	TOTAL PERSON AMOUNT (MG) ABSORBED A	TOTAL PERSON AMOUNT (MG) ABSORBED B	MG/KG ABSORBED A	MG/KG ABSORBED B	AVERAGE AMOUNT ABSORBED MG/KG	AVERAGE AMOUNT (MG/KG) ABSORBED PER HOUR
1	Mixer, Backpack Sup'r	72.6	180	173	1.040	1.221	.014	.017	.016	.005
2	Backpack Sprayer	68.1	"	"	9.016	5.804	.132	.085	.109	.036
3	" "	49.2	"	"	4.585	3.173	.092	.063	.078	.026
4	" "	95.3	"	"	4.206	4.003	.044	.042	.043	.014
5	" "	52.2	"	"	1.800	0.969	.034	.018	.026	.009
6	" "	65.8	"	"	2.752		.042		.042	.014
7	" "	74.9	"	"	7.312	3.270	.098	.044	.071	.024
8	Tractor Sup'r	95.3	245	200	3.077	1.169	.032	.012	.022	.006
9	Tractor Driver	84.0	"	"	3.877	3.088	.046	.037	.042	.012
10	" "	106.7	"	"	4.369	5.766	.041	.054	.048	.014
11	Mixer	79.5	"	"	6.818	4.240	.086	.053	.070	.020
12	Microfoil Pilot	95.3	55	117	0.190	1.151	.002	.012	.007	.005
13	" Mixer	109	"	"	10.130	8.802	.093	.081	.007	.061
14	" Sup'r	84	"	"	0.541	0.455	.006	.005	.006	.004
15	" Flagman	61.3	"	"	0.469		.008		.008	.006
16	" "	74.9	"	"	0.241	.102	.003	.001	.002	.001
17	Raindrop Pilot	72.6	115	116	3.323	3.560	.046	.049	.048	.024
18	" Mixer	86.3	"	"	7.431	13.501	.086	.156	.121	.063
19	" Sup'r	81.7	"	"	0.877	0.153	.011	.002	.007	.004
20	" Flagman	86.3	"	"	0.275	0.112	.003	.002	.003	.002
21	" "	95.3	"	"	0.251	0.345	.003	.004	.004	.002

* A and B represent two exposures.

TABLE A-3

Estimated Occupational Exposure to 2,4,5-T

USE PATTERN	EXPOSED WORKER ACTIVITY	WORKER NUMBER	AVG. AMOUNT ABSORBED (mg/kg/hr)	GROUP AVERAGES (mg/kg/hr)
AERIAL	Pilot - Microfoil	12	.005	.015
"	Pilot - Raindrop	17	.024	
"	Mixer - Microfoil	13	.061	.062
"	" - Raindrop	18	.063	
"	Sup'r - Microfoil	14	.004	.004
"	" - Raindrop	19	.004	
"	Flagman - Microfoil	15	.006	.003
"	" "	16	.001	
"	" "	20	.002	
"	" "	21	.002	
GROUND	Mixer/Loader - Tractor	11	.020	.020
"	Driver - Tractor	10	.014	.013
"	" - "	9	.012	
"	Sup'r - "	3	.006	.006
"	Applicator - Backpack	7	.024	.021
"	" "	6	.014	
"	" "	5	.009	
"	" "	4	.014	
"	" "	3	.026	
"	" "	2	.036	
"	Mixer/Supervisor	1	.005	.005

Table A-4*

TCDD Residues† From Adipose Tissue - Phase One Beef

Application Rate (lb/A)	Sample** Number	ppt 2,3,7,8-TCDD*** (limit of detection)	Application Rate (lb/A)	Sample** Number	ppt 2,3,7,8-TCDD*** (limit of detection)
4	NZZD18	6(6), 8(8), ND(6)	2	KZZD93	ND(3)
4	NZZD19	ND(12)	2	KZZD95	ND(7), ND(8)
			2	KZZD98	ND(13)
3	UZZD02	ND(6)	2	KZZD99	ND(6)
3	UZZD05	10(9), ND(5), ND(12.5)	2	KZZL00	ND(3), ND(5)
3	UZZD06	21(9), 23(5), 29(13), ND(13), ND(20)			
3	UZZD07	ND(10), ND(24)	1	NZZD30	10(10)
3	UZZD10	12(3), 16(6), 39(8), 45(13), 48(5), 54(5), 63(13), 66(13), 75(3), 89(7), ND(4)	1	NZZD61	5(1), 5(2), 7(6), ND(7), ND(10)
			1	NZZD26	ND(6)
3	UZZD11	18(7), ND(3), ND(4), ND(12)	1	NZZD59	ND(9)
3	UZZD13	ND(8), ND(16)	1	NZZD60	ND(14)
3	UZZD14	ND(21)	1	NZZD69	ND(9)
3	UZZD15	14(5), 20(8), ND(3), ND(20)	1	NZZD70	ND(6)
3	UZZD16	8(6), 9(9), 14(10), ND(4), ND(13)			
3	UZZD17	19(12), 20(8), 20(8), 24(20), 29(10)	1/2	NZZD20	ND(2)
			1/2	XZZD72	ND(9)
2	KZZD47	9(5), 22(20), ND(27)	1/2	XZZD73	ND(6)
2	KZZL01	22(14), ND(80)	1/2	XZZD75	ND(6)
2	KZZL02	30(18), ND(20)	1/2	XZZD78	ND(10)
2	KZZD36	ND(6)	1/2	XZZD80	ND(9)
2	KZZD38	ND(17)	1/2	XZZD82	ND(7)
2	KZZD40	ND(10)	1/2	XZZD84	ND(7)
2	KZZD43	ND(20)	1/2	XZZD87	ND(8)
2	KZZD49	ND(13)	1/2	XZZD88	ND(6)

† High resolution MS analyses only.

* This table is modified from Table 1 of the testimony of G. Streisinger (EPA Exhibit No.564). Reported values have been confirmed from the original worksheets.

** Each entry represents a different animal.

*** Analyses were performed on identical extracts of the same sample.

Table A-5

TCDD Residues from Adipose Tissues - Phase Two Beef

Application Rate (lb/A)	Sample Number	ppt. 2,3,7,8-TCDD (Limit of Detection)
3	BAII-4	ND(10)
3	BAII-5	7(7), 8(7)
2	BAII-9	7(7), 11(7)
2	BAII-1	ND(10)
2	BAII-2	ND(10)
2	BAII-6	ND(8)
2	BAII-8	ND(10)
2	BAII-12	13(10), 15(10)
2	BAII-16	ND(8)
2	BAII-17	ND(7)
2	BAII-18	ND(7)
2	BAII-20	31(8), 34(8)
2	BAII-21	ND(10)
2	BAII-22	ND(10)
2	BAII-46	ND(8)
2	BAII-47	ND(10)
3/4	BAII-34	ND(10)
3/4	BAII-35	ND(10)
3/4	BAII-35R	ND(10)
3/4	BAII-36	ND(10)
3/4	BAII-36R	ND(10)
1/2	BAII-10	ND(14)
1/2	BAII-11	ND(10)
1/2	BAII-14	ND(8)
1/2	BAII-23	ND(8)
1/2	BAII-26	ND(7)
1/2	BAII-27	9(7), 10(7)
1/2	BAII-28	ND(8)
1/2	BAII-31	ND(7)

Table A-6

Adjusted Residue Data - Theoretical ppt TCDD - Phase One Beef

lb/A	Sample* Number	Adjusted** Residues (ppt)	lb/A	Sample* Number	Adjusted** Residues (ppt)
3	UZZ002	6.0	1	NZZ030	10
3	UZZ005	9.2	1	NZZ061	4.5
3	UZZ006	21.2	1	NZZ026	2.0
3	UZZ007	17.0	1	NZZ059	3.0
3	UZZ010	46.5	1	NZZ060	4.7
3	UZZ011	9.3	1	NZZ069	3.0
3	UZZ013	12.0	1	NZZ070	2.0
3	UZZ014	21.0		mean =	4.17 ppt
3	UZZ015	14.3			
3	UZZ016	9.6			
3	UZZ017	22.4	1/2	NZZ020	0.33
	mean =	17.1 ppt	1/2	XZZ072	1.50
			1/2	XZZ073	1.00
			1/2	XZZ075	1.00
2	KZZ047	16.3	1/2	XZZ078	1.67
2	KZZ101	37.7	1/2	XZZ080	1.50
2	KZZ102	21.7	1/2	XZZ082	1.17
2	KZZ036	4.0	1/2	XZZ084	1.17
2	KZZ038	11.3	1/2	XZZ087	1.33
2	KZZ040	6.7	1/2	XZZ088	1.00
2	KZZ043	13.3	1/2	XZZ090	1.50
2	KZZ049	8.7		mean =	1.20 ppt
2	KZZ093	2.0			
2	KZZ095	5.0			
2	KZZ098	8.7			
2	KZZ099	4.0			
2	KZZ100	2.7			
	mean =	10.93 ppt			

* Each sample represents a different animal.

** Each numerical entry is the computed mean of all analyses run on that one sample, irrespective of which collaborator generated the value. Non-detected (ND) samples are reported as if the TCDD residues were actually measured according to the following scheme:

a. 4 lb/A (not included in this evaluation due to small sample size)
b. 3 lb/A treatment group, at the limit of detection reported.
c. 2 lb/A treatment group, at 2/3 the limit of detection reported.
d. 1 lb/A treatment group, at 1/3 the limit of detection reported.
e. 1/2 lb/A treatment group, at 1/6 the limit of detection reported.

For additional assumptions, see discussion on page 32.

Table A-7

Criteria Used by the Dioxin Monitoring Program to Confirm TCDD Residues

1. Capillary column GC/HRMS retention time of reference standard 2,3,7,8-TCDD.

2. Co-Injection of sample fortified with ^{37}Cl-TCDD and 2,3,7,8-TCDD standard.

3. Correct molecular ion chlorine isotope ratio (m/e 320 and m/e 322).

4. Capillary column GC/HRMS which give simultaneous multiple ion monitoring response (m/e 320, m/e 322 and m/e 328) for TCDD.

5. M/e 320 and m/e 322 MS response greater than 2.5 x noise level.

6. Recoveries of added TCDD must be between 50 and 120%

Table A-8

ESTIMATED DIETARY INTAKE OF FAT FROM MILK AND DAIRY PRODUCTS

Milk or Dairy Product	gm/person/day*	% fat**	gm fat/day
Fresh Fluid Milk	383.31	4.	15.3
Processed Milk(1)	58.10	8.	4.6
Cream(2)	5.35	28.	1.5
Frozen Milk Desserts(3)	53.14	5.	2.7
Cheese(4)	21.81	29.	6.3
Butter	7.93	81.	6.4
Other	25.58	25.(5)	6.4

Total Dietary Fat Intake = 43 gms fat/person/day.

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* Reference 23.
** Pesticide Analytical Manual, U.S. Food and Drug Administration.

(1) Includes condensed- and evaporated milks.
(2) Includes table- and whipped creams.
(3) Iced Milk
(4) Includes cream-, spread- and swiss cheeses.
(5) Figure given is a mean of the other reported fat levels.

APPENDIX G

THE CARCINOGEN ASSESSMENT GROUP'S

METHOD FOR DETERMINING THE UNIT RISK ESTIMATE

FOR AIR POLLUTANTS

Robert E. McGaughy

For Roy E. Albert, M.D.
Chairman
July 31, 1980

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METHOD FOR DETERMINING THE UNIT RISK ESTIMATE FOR AIR POLLUTANTS

INTRODUCTION

The unit risk estimate for an air pollutant is defined as the lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ug/m^3 of the agent in the air which they breathe. This calculation is done to estimate in quantitative terms the effectiveness (potency) of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air exposure to these agents, if the actual exposures are known. These two uses have different limitations.

In order to use these estimates intelligently, the nature of the source data used and the assumptions necessary to derive the estimates must be clearly understood. This appendix discusses the general approach and the assumptions common to most unit risk estimates. The credence one can ascribe to each risk estimate depends heavily on the quality of the studies on which the estimate is based and the relevance of these studies to the evaluation of air exposure in humans.

PROCEDURES FOR DETERMINATION OF UNIT RISK

The data used for the quantitative estimate is one or both of two types: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses

with an incidence determined by the extrapola· on model discussed below.

A. Choice of Model

There is no really solid scientific basis for any mathematical extrapolation model which relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogens for guidance as to which risk model to use. At the present time the dominant view of the carcinogenic process involves the concept that most agents which cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents which cause cancer are also mutagenic. There is reason to expect the quantal type of biological response that is characteristic of mutagenesis is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenesis studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiological studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxin in the diet). There is also some evidence from animal experiments that is consistent with the linear non-threshold model (e.g., liver

tumors induced in mice by 2-acetylaminofluorene in the large scale ED_{01} study at the National Center for Toxicological Research and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because it has the best, albeit limited, scientific basis of any of the current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be smaller.

The mathematical formulation chosen to describe the linear, non-threshold dose-response relationship at low doses is the improved multistage model developed by Dr. K. Crump. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

B. Description of the Extrapolation Model

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$A(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)},$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, i = 0, 1, 2, ..., k and consequently the extra risk function A(d) at any given dose d is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk A(d) are calculated by using the computer program GLOBAL 79 developed by Crump and Watson (1979). The calculation proceeds as follows: Let Lo be the maximum value of the log-liklihood function. The 95% upper confidence limit for the extra risk A(d) has the form

$$A_u(d) = 1 - \exp\left[-(q_1^* d + \hat{q}_2 d^2 + \ldots + \hat{q}_k d^k)\right]$$

where q_1^* is calculated by increasing q_1 to a value q_1^* such that when the log-liklihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper limit (one-sided). The

quantities $\hat{q}_2$, $\hat{q}_3$, ..., $\hat{q}_k$ are the maximum likelihood estimates of the other coefficients given q_1 equal to q_1^*. This approach of computing the upper confidence limit for the extra risk A(d) is an improvement on the Crump et al. (1977) model. At low doses, the exponent $g(d) = q_1^*d + \hat{q}_2d^2 + \ldots + \hat{q}_kd^k$ is dominated by the linear term q_1^*d and hence

$$A_u(d) = 1 - \exp(-q_1^*d) = q_1^*d$$

Therefore, the upper confidence limit for the extra risk A(d) at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope q_1^* is a measure of the potency of the chemical in inducing cancer at low doses.

In fitting the dose-response model, the number of terms in the polynomial g(d), is chosen equal to (h-1) where h is the number of dose groups in the experiment including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted and the model is refitted to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_iP_i)^2}{N_iP_i(1 - P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever χ^2 is larger than the cumulative 99%

point of the chi-square distribution with f degree of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

SELECTION AND FORM OF DATA USED TO ESTIMATE PARAMETERS IN THE EXTRAPOLATION MODEL

For some chemicals, several studies in different animal species, strains, and sexes each run at several doses and different routes of exposure are available. A choice must be made of which of the data sets from several studies to use in the model. It is also necessary to correct for metabolism differences between species and absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are listed below.

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set which gives the highest estimate of the lifetime carcinogenic risk q_1^* is selected in most cases. However, efforts are made to exclude data sets which produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship and one has a very small sample size, the set of data which has larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size which are identical with respect to species, strain, sex, and tumor sites, the geometric mean of the exponent g(d), estimated from each of these data sets and evaluated at a specific dose d, is used for risk assessment. The geometric mean of numbers

A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study and if the data are available to us, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

4. Following the suggestion of Mantel and Schneiderman (1977) we assume that mg/surface area/day is an equivalent dose between species. Since to a close approximation the surface area is proportional to the 2/3 rds power of the weight as would be the case for a perfect sphere, the exposure in mg/day per 2/3 rds power of the weight is also considered to be an equivalent exposure. In an animal experiment this equivalent dose is computed in the following manner.

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e)

W = average weight of the experimental animal

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

Often exposures are not given in units of mg/day and it becomes necessary to convert the given exposures into mg/day. For example in most feeding studies exposure is in terms of ppm in the diet. In this case the exposure in mg/day is

$$m = ppm \times F \times r$$

where ppm is parts per million in the diet of the carcinogenic agent and F is the weight of the food consumed per day in kgms and r is the absorption fraction. In the absence of any data to the contrary r is assumed to be equal to one. For a uniform diet the weight of the food consumed is proportional to the calories required which in turn is proportional to the surface area or 2/3rds power of the weight, so that

$$m \propto ppm \times W^{2/3} \times r \text{ or}$$

$$\frac{m}{rW^{2/3}} \propto ppm$$

As a result, ppm in the diet is often assumed to be an equivalent exposure between species. However, we feel that this is not justified since the calories/kg of food is very different in the diet of man compared to laboratory animals primarily due to moisture content differences. Instead we use an empirically derived food factor f = F/W which is the fraction of a species body

weight that is consumed per day as food. We use the rates given below.

Species	W	f
Man	70	0.028
Rat	0.35	0.05
Mice	0.03	0.13

Thus, when the exposure is given as a certain dietary concentration in ppm the exposure in $mg/W^{2/3}$ is

$$\frac{m}{r \times W^{2/3}} = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times fxW}{W^{2/3}} = ppm \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = m/Wr = s the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}$$

When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water soluble gas or an aerosal and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water soluble gas which reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

Case 1

Agents that are in the form of particulate matter or virtually completely absorbed gases such as SO_2 can reasonably be expected to be absorbed proportional to the breathing rate. In this case the exposure in mg/day may be expressed as

$$m = I \times \quad \times r$$

where

I = inhalation rate per day in m^3

v = mg/m^3 of the agent in air

r = the absorption fraction

The inhalation rates, I, for various species can be calculated from the observations (FASEB 1974) that 25 gm mice breathe 34.5 liters/day and 113 gm rats breathe 105 liters/day. For mice and rats of other weights, W (in kilograms), the surface area proportionality can be used to find breathing rates in m^3/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

For humans, the values of 20 m^3/day† is adopted as a standard breathing rate (ICRP 1977).

The equivalent exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in way analogous to the food intake data.

†From "Recommendation of the International Commission on Radiological Protection", page 9, the average breathing rate is 10^7 cm^3 per 8 hour work day and 2×10^7 cm^3 in 24 hours.

The empirical factors for the air intake per kg per day, i = I/W based upon the previous stated relationships are tabulated below

Species	W	i = I/W
Man	70	0.29
Rat	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

$$\frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}\,vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

Case 2

The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption which in turn is proportional to $W^{2/3}$ and also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficiennt r for the gas. Therefore, expressing the O_2 consumption as $O_2 = kW^{2/3}$ where k is a cconstant independent of species, it follows that

$$m = k\,W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

As with Case 1, in the absense of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain

concentration in ppm or ug/m^3 in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration that is necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps, et al. 1975). When the animals were exposed via the oral route and human exposure is via inhalation or vice-versa, the assumption is made, unless there is pharmacokenetic evidence to the contrary, that absorption is equal by either exposure route.

5. If the duration of experiment (Le) is less than the natural lifespan of the test animal (L), the slope q_1^* or more generally the exponent g(d) is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose, D, is continued, the age specific rate of cancer will continue to increase as a constant function of the background rate. The age specific rates for humans increases at least by the 2nd power of the age and often by a considerably higher power as demonstrated by Doll (1971). Thus, we would expect the cumulative tumor rate to increase by at least the 3rd power of age. Using this fact we assume that the slope q_1^* or more generally the exponent g(d), would also increase by at least the 3rd power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age Le, we would expect that if the experiment had been continued for the full lifespan, L, at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/Le)^3$.

This adjustment is conceptually consistent to the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Crump et al. (1979) where the probability of cancer at age t and dose d is given by

$$P(d,t) = 1 - \exp[-f(t) \times g(d)]$$

This more refined approach would be used in the calculations of unit risk wh n the data are available.

CALCULATION OF OF THE UNIT RISK

The risk associate with d mg/kg$^{2/3}$/day as noted previously is

$$A_u(d) = 1-\exp[-(q_1^* d + \hat{q}_2 d^2 + \ldots + \hat{q}_k d^k)]$$

A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. To estimate this value we simply find the number of mg/kg$^{2/3}$/day corresponding to one unit of X and substitute this value into the above relationship. Thus, for example if X is in units of ug/m^3 in the air we have that for

$$\text{case (1)} \quad d = 0.29 \times 70^{1/3} \times 10^{-3} = 1.195 \times 10^{-3}$$

and for case (2) d = 1 when ug/m^3 is unit used to compute parameters in animal experiment.

If exposures are given in terms of ppm in air we may simply use the fact that

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3$$

Note, an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures and then increase the jth polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \qquad j = 1,2,\ldots,k$$

and use mg/kg equivalents for the unit risk values.

ESTIMATION OF UNIT RISK BASED ON HUMAN DATA

If human epidemiology studies and sufficiently valid exposure information are available for the compound, they are always used in some way. If they show a carcinogenic effect, the data are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose, which is equivalent to the factor B_H. If they show no carcinogenic effect when positive animal evidence is available, then it is assumed that a risk does exist but it is smaller than could have been observed in the epidemiology study, and an upper limit of the cancer incidence is calculated assuming hypothetically that the true incidence is just below the level of detection in the cohort studied, which is determined largely by the cohort size. Whenever possible, human data are used in preference to animal bioassay data.

In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals compared to the control group. In the analysis of this data it is assumed that the excess risk, or relative risk minus one, $R(X_1) - 1$, is proportional to the lifetime average exposure, X_1, and that it is the same for all ages. It follows that the lifetime risk in the general population exposed to a lifetime average concentration X_2, $P(X_2)$, is equal to $[R(X_1) - 1]X_2/X_1$ multiplied by the lifetime risk at that site in the general population. The unit risk estimate is the value of P when X_2 is 1 ug/m^3. Except for an unusually well documented human study, the confidence limit for the excess risk P is not calculated, due to the difficulty of accounting for the uncertainty inherited in the data (exposure and cancer response).

INTERPRETATION OF UNIT RISK

The unit risk estimate is a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex and by the same route of exposure, preferably by inhalation.

For several reasons the unit risk estimate is only an approximate indication of the absolute risk in populations exposed to known air concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Secondly, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

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