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September 12, 1980

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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

Office of the General Counsel
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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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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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(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

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.

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.

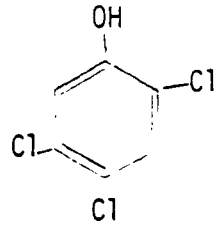
Two cohort studies, one by Axelson et al. (1980) and the other by Thiess and Frentzel-Beyme (1977) provide suggestive evidence that phenoxyacetic acids and/or TCDD increases the risk of stomach cancer in humans.

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

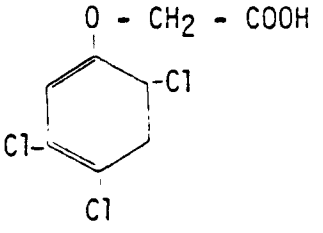
The assessment of risk from TCDD exposure covers only the herbicide applicators and dietary exposure to beef, milk, deer, and elk. For unprotected workers, the upper limits of lifetime risk of induced cancers are in many cases as high as or in the 10^{-3} range. For the general population exposed to 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.

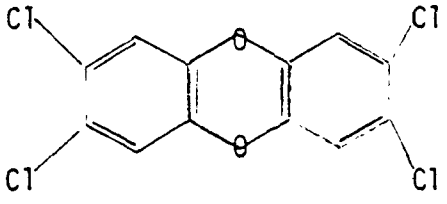
The structure of the four compounds is shown in Figure 2 below.



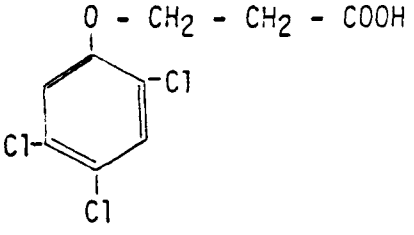
2,4,5-trichlorophenol
(2,4,5-TCP)



(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.

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.

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

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

III. MUTAGENICITY

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

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

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

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.

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).

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

V. CARCINOGENICITY

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"

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.

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.

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.

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 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).

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.

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

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

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

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

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.

CARCINOGENICITY OF TCDD IN RATS AND MICE

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

female rats at doses of 0.1 and 0.01 ug/kg/day (2200 and 220 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.

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 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

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

Type of tumor	Vehicle control	Low Dose[a] 0.01	Mid Dose[a] 0.05	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.

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.

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

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

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.

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.

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.

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%.

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.

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 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.

demonstrated th enhancement by TCDD of tumor induction when TCDD was administered s· ıultaneously 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).

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.

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.

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.

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.

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.

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

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 Observed	Expected	Relative risk	90% confidence interval
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

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

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.

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.

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

$$P = 1 - e^{-B_1 x} = 1 - e^{-0.017x}$$

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

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

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.

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 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 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 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 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 50. 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.....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 X^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 52. 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.....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	1×10^{-6}	1×10^{-5}	1×10^{-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	1.25×10^4	0	0		14.47 (d.f.=2)
When the highest dose group is not used	0.19	0	5.83×10^9		7.90×10^4	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 ($P > 0.01$) = 7.90×10^4

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

TABLE 54. 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.....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 56. 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.....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 X^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 58. 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 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}

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

TABLE 60. LIFETIME PROBABILITY OF INDUCED CANCER FOR 2,4,5-T AND SILVEX APPLICATORS BASED ON 2,4,5-T MEASURED EXPOSURE[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 mg/kg/year for 40 years = 40 year $\times \frac{1 \text{ life}}{71.3 \text{ years}} \times \frac{1 \text{ year}}{365 \text{ days}} = 1.54 \times 10^{-3}$ 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. (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 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.

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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-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-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)

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 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
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	

ROBERT A. SQUIRE ASSOCIATES, INC.
1515 LeSelle Avenue
Ruxton, Maryland 21204

(301) 821-0054

August 26, 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 FÜR PHARMAKOLOGIE UND TOXIKOLOGIE
PROFESSOR DR. P. 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:

		2,4,5-T (untreated rats)	2,4,5-T (acetone-treated rats)
A)	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

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. 1083/210 223

D) The tongue was examined microscopically 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 T)XIKOLOGIE
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

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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.

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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APPENDIX E

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.

APPENDIX F

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

EPA Form 1320-6 (Rev. 3-76)

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.

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,

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

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.

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.

---

* 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.

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®, was applied at day "0" at a rate of 2 lbs a.e./A*

---

* 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

...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 <u>average experimental dose</u> 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

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	Applicators (hand) Basal	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.

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-HEDMAN 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 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.

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.

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.

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

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

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.

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

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

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.

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).

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.

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.

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),

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 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

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-

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.

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,

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.

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.

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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

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

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.

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. 5-118)

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.

b. Cut Stump (calculation Summary 9)

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.)

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-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-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-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%

APPENDIX G

THE CARCINOGEN ASSESSMENT GROUP'S

METHOD FOR DETERMINING THE UNIT RISK ESTIMATE

FOR AIR POLLUTANTS

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FOR Roy E. Albert, M.D.
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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

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

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

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

$$m \alpha ppm \times W^{2/3} \times r \text{ or}$$

$$\frac{m}{rW^{2/3}} \alpha ppm$$

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

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.

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

$$P(d,t) = 1 - \exp[-f(t) \times g(d)]$$

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).

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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 ...

Compound	Species	Study	Sex	Pathologist	Human Slope Estimate q_1^*
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

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