HEALTH IMPLICATIONS OF 2,3,7,8-TETRACHLORO-DIBENZODIOXIN (TCDD) CONTAMINATION OF RESIDENTIAL SOIL

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Extrapolations from animal toxicity experiments (including carcinogenicity and reproductive effects) to possible human health effects can be used to estimate a reasonable level of risk for 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD). Extrapolations are derived from: (1) review of published studies, (2) a complex set of assumptions related to human exposure to contaminated soil, and (3) estimates of (a) a dose response curve, (b) appropriate margins of safety, and/or (c) applicable mechanisms of action.

One ppb of 2,3,7,8-TCDD in soil is a reasonable level at which to begin consideration of action to limit human exposure for contaminated soil.

SUMMARY

From the available literature dealing with the toxic effects of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), only reports on a few chronic feeding studies in rodents can be used for risk assessment calculations. The smallest lower confidence bound on the virtually safe dose by the linear derived multistage model using an added cancer risk of 1/1,000,000 is calculated to be 28 fg/kg body weight·d (body weight = b.w.). This

47

calculation is based on data for hepatocellular carcinoma or neoplastic nodules. The increased cancer risk of 1/1,000,000 based on data for tissue less sensitive than liver would not be expected to occur until doses as high as 1428 fg/kg b.w.·d were administered. The corresponding levels for an increased risk of 1/100,000 are 276 fg to 14.3 pg/kg b.w.·d (Figs. 1 and 2). These calculations assume that a linear dose-response relationship exists for carcinogens (such as TCDD) that, based on current evidence, are thought to be primarily promoters. However, the dose-response curve for promoters may not be linear, causing an overestimate of the risk. The model was used on a hypothetical basis, and the cancer risk for TCDD should be reevaluated as the data base enlarges. Human exposure would primarily occur by the dermal and the oral route.

To estimate human TCDD intake after exposure to TCDD-contaiminated soil in residential areas, we calculated estimates for dermal, ingestion, and inhalation doses. With these estimates (the assumptions on which they are based are outlined in the text, the best estimate of a daily dose at 1 ppb in residential soil (assuming uniform distribution of TCDD in soil at 1 ppb) is calculated to be 44.6 pg/d (or 636.5 fg/kg b.w.·d for a person weighing 70 kg). In consideration of the range of the estimated VSD and because of the unlikelihood that all of the conservative exposure assessment assumptions will be realized on a continuous or lifetime basis, we have concluded that residential soil levels greater than 1 ppb TCDD pose a level of concern. The appropriate degree of concern for which management decisions are made should also consider an evaluation of the specific circumstances at each contaminated site.

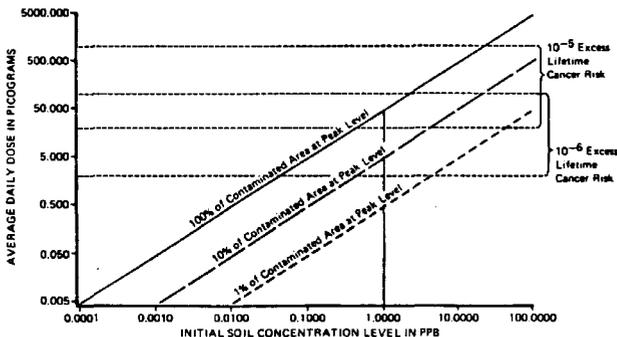Exposure in contaminated residential areas would be greater than in

FIGURE 1. This figure illustrates the average daily dose of TCDD that would be received if 100, 10, or 1% of the accessible soil were contaminated with the peak recorded level of TCDD. The boundaries for an excess lifetime cancer risk for 10^{-5} and 10^{-6} are also illustrated.
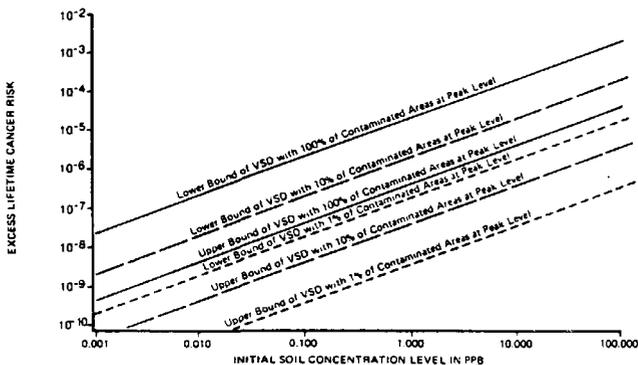
FIGURE 2. This figure illustrates the lower and upper bounds of the virtually safe dose for a lifetime excess cancer risk. This information was derived from the calculations developed from animal data (Tables 8 and 9). At the concentration of 1 ppb, if 100% of the accessible soil contained TCDD at this concentration, the area of risk bordered by the upper and lower bound of the virtually safe dose does not represent an unacceptable cancer risk given the fact that the background cancer incidence in the general population is of a much higher order of magnitude. If less than 100% of the soil was contaminated, this risk would even be further reduced. However, at levels much above 1 ppb the risk would become unacceptable.

only occasionally frequented commercial areas. In residential areas, levels at or above 1 ppb TCDD in soil cannot be considered safe and represent a level of concern. In certain commercial areas, higher levels may present an acceptable risk to nonoccupationally exposed individuals. On ranges and pastures, however, lower soil levels may still be of concern, since TCDD accumulates in the tissues of grazing cattle and rooting swine.

BACKGROUND

In the early 1970s, a waste-oil dealer in Missouri disposed of waste material containing 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in high concentrations by mixing this material with salvage oil and spraying it on dirt roads and riding arenas. The contamination of the riding arenas by TCDD was established in 1975 (Carter et al., 1975). Until recently, investigators had not realized the extent to which several other areas, many of them residential, were also contaminated with TCDD. Thus far, concentrations measured in soil in these areas have ranged from less than 1 ppb to over 1000 ppb. Once it was determined that TCDD was present in residential areas, it had to be decided what level represented an unacceptable risk to the population living in these contaminated areas. This document presents

a detailed review of the initial determination made by the Centers for Disease Control (CDC) and includes suggestions and comments on the report made by a group of consultants. Where appropriate, consultants' comments have been directly incorporated in the document.

The method used for conducting a risk assessment and identifying a level of concern for TCDD in soil is complicated by many uncertainties—namely, insufficient data on the toxicology of TCDD, insufficient information about exposure of people to soil, and insufficient information about intake of TCDD by humans from such soil.

Since ambient concentrations of TCDD in water or air will usually not make a significant additional contribution to TCDD intake for populations living in significantly contaminated areas, we have not addressed these contributions in this document.

INTRODUCTION

Much has been written about TCDD, and it is not the purpose of this document to review all available literature. For an overview, the reader is referred to recent reviews (Kimbrough, 1980; National Research Council of Canada, 1981). Primarily, toxicology information useful for risk assessment will be reviewed here. In making risk assessments, investigators must consider the possible routes of human exposure and the average daily dose or the total lifetime dose. Although some adverse health effects have been observed in humans after exposure to TCDD, the dose these individuals received was not quantified. The only available dose-response data were obtained in animal studies. A critical feature of TCDD is that some animal species are much more susceptible to the toxic effects of TCDD than others. Since no dose-response information is available from human exposures, it is not known which animal species most nearly approximates human responses, although it appears that humans may not be the most sensitive species. From experience with other substances, the guinea pig is often more sensitive than most other species.

Some scientists have even claimed that, with all of the exposure humans have received in occupational situations, humans must not be very susceptible to the toxic effects of TCDD, and the main health effect in humans is chloracne (May, 1973). Such far reaching statements seem inappropriate, however, since no information is available on how much TCDD the exposed workers who developed chloracne actually absorbed systemically and since chloracne can be either (1) part of a systemic disease or (2) produced locally by applying TCDD to the skin, with no toxic levels being absorbed into the body (Bauer et al., 1961). It is also too early to conclude what the long-term effects of chronic low-level exposure in humans will be. Furthermore, in estimating the risk that TCDD poses for the general population, we must consider children, females of childbearing age, the aged, and the infirm.

When given as a single dose, TCDD has a half-life of 12–30 d in small laboratory animals. After repeated dosing, it accumulates in the body and is stored in adipose tissue and, to some extent, in the liver and other organs. The study by Kociba et al. (1978) indicates that at some dosage levels the rat has higher levels in the liver than in adipose tissue. *In vivo* and *in vitro* studies with radiolabeled TCDD demonstrate that the substance is not readily metabolized by cells. In animal studies, however, unidentified metabolites of TCDD have been found in urine and bile, with the high-pressure liquid chromatography (HPLC) elution time of the metabolite varying in different species (Neal et al., 1982).

TCDD produces a multitude of toxicological effects. These effects are reviewed in the articles listed under "Selected References." TCDD is extremely toxic (oral LD50 less than 5 mg/kg b.w.) in most species tested. The effect of TCDD is delayed—animals given a single dose may not die until 40 d later. With repeated dosing, TCDD is toxic at much lower daily dosage levels, and the toxic effects of TCDD appear to be cumulative. From a risk assessment standpoint, fetotoxicity and reproductive dysfunction caused at very low dosage levels in rodents and subhuman primates and carcinogenicity in rodents are critical. Long-term feeding studies are available for these two toxicological end points in rodents. On the other hand, no long-term studies have been made to determine what the no-observed effect level for immunotoxicity is in the monkey, the rat, or the guinea pig, nor have cancer studies been conducted in species other than rodents.

In a 2-yr feeding study in rats (Kociba et al., 1978), the no-observable-effect level was 0.001 μg/kg·d. In one study the oral LD50 in rats was reported as 44 μg/kg. The ratio between the oral LD50 and a long-term daily no-effect level in rats is 44,000. If this same ratio were applied to guinea pigs, and if the oral LD50 in guinea pigs of 0.6 μg/kg is used, a no-effect level for chronic studies in guinea pigs might be calculated to be 0.016 ng/kg·d. A similar calculation can be made by using data obtained with monkeys (the lowest effect level is used here, since a chronic no-effect level has not been determined). The LD50 is from McConnell et al. (1978), and the effect level is from Allen et al. (1979):

$$\frac{\text{LD50}}{\text{Effect level}} = \frac{50\text{–}70 \ \mu g/kg}{1.8 \times 10^{-3} \ \mu g/kg \cdot d} = 27{,}700\text{–}38{,}888$$

TCDD is highly lipophilic, degrades rapidly on exposure to ultraviolet light if hydrogen donors are available, does not readily migrate through soil, and appears to be only slightly taken up by root plants; furthermore, only a few strains of soil bacteria can degrade it, at a very slow rate. The half-life of TCDD in soil is not known. The earlier reports stating that its half-life is less than a year appear to be erroneous.

The concentrations at which TCDD still causes toxic effects are

difficult to imagine. For instance, $1 \mu g = 10^{-6}$ g, $1 ng = 10^{-9}$ g, $1 pg = 10^{-12}$ g, and $1 fg = 10^{-15}$ g. There are 28 g in 1 ounce. One molecule of TCDD weighs

$$\frac{322 \text{ g/mol}}{6.023 \times 10^{23} \text{ molecules/mol}} = 5.35 \times 10^{-22} \text{ g/molecule}$$

This translates into 1.8×10^6 molecules/fg.

SOURCES AND OCCURRENCE OF TCDD

In the production of 2,4,5-trichlorophenol from tetrachlorobenzene, TCDD is formed as a contaminant. Subsequent clean-up of 2,4,5-trichlorophenol results in industrial waste (still-bottom residue) that contains high (up to 1000 ppm) concentrations of TCDD. The products made from 2,4,5-trichlorophenol, such as 2,4,5-T (trichlorophenoxyacetic acid) and hexachlorophene, and 2,4,5-trichlorophenol itself, may still be contaminated with trace amounts of TCDD.

TCDD and other chlorinated dibenzodioxins may also be formed during combustion. These chemicals have been identified in soot, fly ash, and many other products that were burned (Bumb et al., 1980; Buser et al., 1978). The concentrations found have usually been in parts per trillion (nanogram per kilogram) (Eiceman et al., 1980; Kooke et al., 1981).

Because of the inherent toxicity of TCDD, levels in the environment at concentrations in the parts per trillion to parts per billion range may be of toxicological significance. Methods have only recently been developed to measure such low concentrations. Therefore, no systematic monitoring for TCDD in the environment has been conducted (Norstrom et al., 1982).

Soil levels measured recently in contaminated sites in Missouri have ranged from less than $1 \mu g/kg$ (ppb) to over 1 mg/kg (ppm) [Regional U.S. Environmental Protection Agency (EPA), Kansas City, Missouri, unpublished information]. Concentrations measured in contaminated riding arenas in 1971 and 1974 were 30 mg/kg and after excavation around 1 mg/kg (Carter et al., 1975). EPA is now characterizing soil TCDD levels at sites where 2,4,5-trichlorophenol was produced or used to make 2,4,5-T and other chemicals (Anonymous, 1983).

METABOLISM IN ANIMALS

The toxicokinetics are reviewed by Neal et al. (1982).

Absorption

In Sprague-Dawley rats given a single oral dose of 1.0 μg ^{14}C-labeled 2,3,7,8-TCDD/kg b.w., absorption from the intestinal tract was estimated to be around 83% (Rose et al., 1976). With repeated oral dosing at 1.0

μg/kg·d (5 d/wk × 7 wk), absorption was similar to that observed for the single oral dose (Rose et al., 1976). With a much larger single oral dose, 50 μg/kg b.w., about 70% of the dose was absorbed by rats (Piper et al., 1973). In studies where TCDD was administered to rats by gavage in acetone:corn oil (1:25 or 1:9), absorption from the gastrointestinal tract ranged from 70–83% (Rose et al., 1976; Piper et al., 1973). When TCDD was administered to rats in the diet at 7 or 20 ppb (0.5 or 1.4 μg/kg·d) for 42 d, 50–60% of the consumed dose was absorbed (Fries and Marrow, 1975).

Poiger and Schlatter (1980), using hepatic concentrations in rats 24 h after dosing as an indicator of the amount absorbed, found a linear relationship between nanograms of TCDD administered in 50% ethanol (for doses of 12–280 ng, equivalent to 0.06–1.4 μg/kg) and the percentage of the dose in hepatic tissues (36.7–51.5%). Only about half of the amount of TCDD given in ethanol was absorbed when TCDD was administered in an aqueous suspension of soil.

Information on the absorption of 2,3,7,8-TCDD through the skin is found only in a study by Poiger and Schlatter (1980), and no information on absorption through the respiratory tract was found. Poiger and Schlatter (1980) administered 26 ng TCDD in 50 μg methanol to the skin of 6 rats. After 24 h, the liver contained $14.8 \pm 2.6\%$ of the dose. Application of TCDD in a soil/water paste decreased hepatic TCDD to about 2% of the administered dose at a dose of 350 ng and to about 0.05% at a dose of 26 ng. It is not clear whether absorption from the skin would be as rapid as that from the stomach. These studies suggest that small amounts of TCDD are absorbed through the skin, but they give no information about the rate of absorption and distribution of TCDD in the body over time.

Distribution

Piper et al. (1973) used a single oral dose of [^{14}C]TCDD to study distribution and excretion of TCDD in male Sprague-Dawley rats. Most of the radioactivity (53.2%) was excreted via the feces, but the urine and expired air accounted for 13.2 and 3.2%, respectively. Analysis of the tissues after 3 d showed liver and adipose tissue to contain the highest percentage of the dose per gram of tissue, with 3.18 and 2.60%, respectively.

Rose et al. (1976) also examined the distribution of [^{14}C]TCDD in rats. At 22 d after a single oral dose of 1.0 μg/kg, liver and adipose tissue had each retained about 1.2% of the dose. With repeated oral doses of 0.01, 0.1, or 1.0 μg [^{14}C] TCDD·kg/d, Monday through Friday for 7 wk, the liver and fat contained most of the body burden of TCDD, accounting for 50 and 10 times more ^{14}C activity, respectively, than the rest of the carcass. As a result of these studies, they concluded that the rate of TCDD accumulation in the body, after single and repeated exposure, was largely

accounted for by the rate of accumulation in liver and fat. With a single oral dose, no radioactivity was detected in either the urine or expired air, indicating that most, if not all, of the elimination of TCDD and/or its metabolites was via the feces. With repeated oral doses, the ^{14}C activity was also excreted primarily through the feces, but significant amounts were found in the urine. Male rats given 1.0 µg TCDD/kg·d for 7 wk excreted an average of 3.1% of the cumulative dose in the urine, whereas the female rats excreted an average of 12.5% in the urine (Rose et al., 1977).

Studies performed by Van Miller et al. (1977) on rhesus monkeys and rats with single intraperitoneal doses of tritiated TCDD showed that although rats had over 40% of the TCDD in the liver 7 d after dosing, the monkeys had only about 10% in the same organ at that time, and a greater percentage was found in skin and muscle tissue. In two strains of mice, the liver contained about 35% of an administered dose of TCDD 1 d after oral or intraperitoneal administration (Manara et al., 1982).

Kociba et al. (1978) found that female rats maintained on a daily dietary TCDD intake of 0.1 µg/kg·d for 2 yr had an average TCDD content of 8100 ppt (ng/kg) in fat and 24,000 ppt (ng/kg) in the liver. Rats given 0.01 µg/kg·d had an average of 1700 ppt (ng/kg) TCDD in the fat and 5100 ppt (ng/kg) in the liver. For both of these daily dosages, the liver-to-body-fat ratio of TCDD was 3 to 1. At the lowest dose level of 0.001 µg/kg·d, both fat and liver contained an average of 540 ppt 2,3,7,8-TCDD. Kociba et al. (1978) presented evidence that a steady state in rats had been reached after 13 wk of TCDD feeding.

McNulty (1982) reported that 2 yr after administering a single oral dose of 1 µg TCDD/kg to an adult rhesus macaque monkey, tissue levels of the compound were 100 ppt (ng/kg) in adipose tissue and 15 ppt (ng/kg) in liver. These results indicate that prolonged retention of TCDD may occur in this species. The retention in humans is not known.

TCDD has also been shown to pass the placenta of rats and mice (Moore et al., 1976; Nau and Bass, 1981; Applegren et al., 1983), and it is excreted in milk (Moore et al., 1976).

TCDD is a potent inducer of hepatic microsomal mixed-function oxidase enzymes with the lowest effective single dose in the rat of 0.002 µg/kg b.w. (Kitchin and Woods, 1979).

Excretion

The following discussion assumes that elimination is a first-order process, except in the guinea pig, in which elimination may follow zero-order kinetics (Gasiewicz and Neal, 1979).

TCDD is slowly excreted from the bodies of all small laboratory species tested, with a half-life in the body for single doses of approximately 10–31 d. In the golden Syrian hamster, the mammalian species least

sensitive to the acute toxicity of TCDD, excretion occurs readily through both the urine (41% of total excreted radioactivity) and feces (59% of total excreted radioactivity) (Olson et al., 1980). In all other species tested so far, excretion occurs mainly through the feces, with only minor amounts of TCDD metabolites found in the urine (Rose et al., 1976; Gasiewicz and Neal, 1979).

Metabolites of TCDD have been detected in the bile and urine of golden Syrian hamsters after single oral or intraperitoneal doses (Olson et al., 1980) and in the bile of dogs after repeated direct introduction of the chemical into the duodenal lumen (Poiger et al., 1982).

Poiger and Schlatter (1980) and Ramsey et al. (1979) demonstrated biliary excretion of several metabolites of [^{14}C]TCDD by rats after repeated oral dosing. The metabolites were tentatively identified as glucuronides of hydroxylated TCDD. The amounts of metabolites found were small.

Mutagenesis and Cell Transformation

The results of *in vitro* and *in vivo* mutagenesis studies are summarized by Kociba and Schwetz (1982), Hay (1982), and Rogers et al. (1982). In tests with *Salmonella* strains, TCDD has been reported to be mutagenic in the *Salmonella typhimurium* strain TA 1532 and the *Escherichia coli* strain Sd-4. In other laboratories, strains TA 1532, TA 1535, TA 1537, and TA 1538 have not yielded positive results. On the other hand, prophage induction in *E. coli* K-39 was positive, but a dominant lethal study in rats was not. Cytogenetic studies in rat bone marrow were negative or questionable.

Hay (1982) recently was able to show cell transformation in kidney hamster cells. Although in a number of cell lines it was possible to induce aryl hydrocarbon hydroxylase, there have been almost no observations of cell toxicity. Only in a mouse teratoma cell were Knutson and Poland (1982) able to induce keratinization by the addition of TCDD. A similar transformation occurs in sebaceous glands when chloracne develops and the cells of the sebaceous glands are transformed into squamous cells.

In many instances there appears to be an association between such metaplasia and the development of cancer. However, metaplasia—that is, cell transformation—may also occur without necessarily progressing into cancer, such as the transformation of columnar into squamous epithelium. Therefore cell transformation per se does not represent conclusive evidence that TCDD is an initiator of carcinogeneis. For instance, in vitamin A deficiency, squamous metaplasia develops in the trachea, bronchus, and the pelvis of the kidneys, the uterus and the pancreatic ducts (Pinkerton, 1977). Although vitamin A has been claimed to protect against the development of certain carcinomas, its lack can certainly not be considered to be an initiator of cancer.

Mechanism of Action

It is not clear why some species are more susceptible to the toxic effects of TCDD and why the target organs vary in different animal species. The toxicity of this chemical apparently depends on the fact that the lateral positions of the molecule are occupied by chlorine, or bromine in the case of brominated compounds (Poland and Glover, 1973).

Induction of hepatic aryl hydrocarbon hydroxylase (AHH), cytochrome P-448, and a number of other enzymes appears to be controlled by a single gene in the mouse known as the *Ah* locus (Poland and Glover, 1975). In inbred strains of mice, AHH inducibility is inherited as a simple autosomal dominant trait. Poland et al. (1976) observed a small pool of displaceable high-affinity binding sites in the hepatic cytosol of rats and mice that has the *in vitro* binding properties predicted for a receptor for induction of AHH. Polycyclic hydrocarbons such as 3-methylcholanthrene compete with TCDD for the binding site, but compounds that do not induce this enzyme (throxine, steroids, phenobarbital, DDT) do not compete. As would be expected if this binding site represents the receptor, the displaceable binding of [^{3}H]TCDD to the hepatic cytosol of genetically responsive mice is much greater than that of nonresponsive mice. Recent work indicates that the receptor binds TCDD, and the complex translocates to the nucleus (Greenlee and Poland, 1979). Using isoelectric focusing, Gustaffson and co-workers (Carlstedt-Duke et al., 1978) have recently demonstrated the presence of a protein in hepatic cytosol with a molecular weight of 136,000 after partial digestion with trypsin, which has a high specific binding affinity for TCDD (10^{-9}) similar to that reported by Poland et al. (1976). This protein could be detected in AHH-responsive mice, but not in nonresponsive mice.

All of these data are consistent with the hypothesis that chlorinated dibenzodioxins and polycyclic aromatic hydrocarbons combine with a cytosolic receptor, which enters the nucleus and produces coordinate induction of a number of enzymes. In the rat, mouse, and a number of other species, these enzymes include AHH and other cytochrome P-448-associated monoxygenase activators, glucuronyl transferase, DT diaphorase, ornithine decarboxylase, and aldehyde dehydrogenase. The nature of the proteins controlled by the genome would depend on the tissue and on the species. Recent studies of *in vivo* covalent binding in rats (Poland and Glover, 1979) demonstrate that covalent binding to DNA (6 pmol TCDD/molecule nucleotide residue) is 4 to 6 orders of magnitude lower than that of most chemical carcinogens, and the binding of DNA is equivalent to 1 molecule TCDD/DNA of about 35 cells. TCDD-induced oncogenicity is, therefore, most likely not caused through a mechanism of covalent binding to DNA and somatic mutation.

It has thus far not been conclusively demonstrated that all toxic effects, specifically lipid peroxidation, are mediated through the cytosol

receptor. A number of related aromatic compounds, such as certain isomers of the halogenated naphthalenes, biphenyls, and dibenzofurans, have similar biological activities if the lateral positions are occupied by chlorine or bromine (Goldstein, 1980).

ANIMAL TOXICOLOGY

Teratogenesis and Reproduction

TCDD is a teratogen in several strains of mice at doses much lower than most other teratogens. Cleft palate and kidney anomalies predominate. When CF-1 mice (between 14 and 41 mice/dose group) were dosed by gavage on d 6-15 of gestation with 5 doses of TCDD ranging from 0.001 to 3 μg TCDD/kg b.w.·d, no significant effects were observed at any dosage of TCDD on implantation sites per litter, live fetuses per litter, sex ratio, fetal body weight, fetal crown-rump length, or skeletal abnormalities. The percent of resorptions increased significantly ($p < 0.05$) at doses of 1 μg TCDD/kg b.w.·d. Cleft palate increased significantly at 1 and 3 μg TCDD/kg b.w.·d, and the incidence of bilaterally dilated renal pelvis was significantly increased at 3 μg TCDD/kg b.w.·d (Smith et al., 1976). The authors concluded that the rate of malformations in CF-1 mice was not significantly increased at doses less than or equal to 0.1 μg TCDD/kg b.w.·d.

In rats, TCDD produces fetotoxic effects at lower doses than are required for teratogenic effects and increases the incidence of intestinal hemorrhage and edema, kidney anomalies, and internal hemorrhage (Murray et al., 1979).

Murray et al. (1979) studied the effects of 0, 0.001, 0.01, and 0.1 μg TCDD/kg b.w.·d given in the diet of Sprague-Dawley rats over three generations. No significant toxic effects were observed in the F_0 generation during 90 d treatment before mating. Both fertility and neonatal survival were significantly reduced in the F_0 generation, and neonatal survival was reduced in the F_1 generation at doses of 0.1 μg TCDD/kg b.w.·d. At 0.01 μg TCDD/kg b.w.·d, fertility was significantly reduced in the F_1 and F_2 generations but not in the F_0 generation. In addition, daily doses of 0.01 μg TCDD/kg b.w.·d reduced litter size, decreased fetal and neonatal survival, and decreased growth. Doses of 0.001 μg TCDD/kg b.w.·d had no effect on fertility, litter size, postnatal body weight, or neonatal survival. Therefore, Murray et al. (1979) concluded that doses of 0.1 and 0.01 but not 0.001 μg TCDD/kg b.w.·d produced deleterious reproductive effects through three generations of rats. Nisbet and Paxton (1982) in their review of these data concluded that the lowest dose still affected reproduction. We concluded that these data could not be used for risk assessment calculations for the following reasons:

The fertility index in the controls and exposed rats varied greatly. It

ranged from 44–88% among the controls, from 50–100% in rats receiving 0.001 μg TCDD/kg b.w.·d, from 55–75% in the rats receiving 0.01 μg/kg b.w.·d. (Fertility index is the number of females delivering a litter divided by the number of females placed with a male.) Such variation in the fertility index is occasionally observed in reproduction studies. Usually the reason why fertility suddenly drops cannot be explained. Such an effect is random and may affect different experimental groups at different times. Once fertility is affected, the number of litters, the number of rats per litter, and the number of dead fetuses may also be affected. During lactation rodents will mobilize persistent chlorinated compounds such as TCDD from adipose tissue and the dose that the offspring receives in milk is much higher than what the dose was that the dam received. Rodents may eliminate more of their body burden through lactation than humans because of the different composition of the milk. Different generations can therefore not be combined.

Khera and Ruddick (1973) observed dose-related decreases in average litter size and pup weight of Wistar rats treated with doses greater than 0.25 μg TCDD/kg b.w.·d. Survival was significantly decreased at 0.5 μg TCDD/kg b.w.·d.

Allen et al. (1979) have reported adverse effects of TCDD on reproduction in nonhuman primates (rhesus monkeys). A decrease in serum estradiol and progesterone levels was noted after a 7-mo exposure to diets containing 500 ng TCDD/kg (approximately 18 ng/kg b.w.·d) and only three of 8 dosed females conceived; of these, 2 aborted.

In a second experiment, Allen et al. (1979) fed female rhesus monkeys a diet containing 50 ng TCDD/kg (approximately 1.8 ng/kg b.w.·d). Mating of 8 treated females with untreated males resulted in 6 pregnancies, from which there were 4 abortions and 2 normal births. All 8 control females conceived and had normal births. This daily dose of 1.8 ng/kg would be equivalent to a total dose of 378 ng/kg b.w.

Allen et al. (1979) and McNulty (1982) studied the effect of TCDD in rhesus monkeys. Whereas Allen et al. (1979) produced effects on reproduction in monkeys with a total dose of roughly 500 ng/kg given over a 6-mo period, McNulty observed similar effects with a single dose of 1 μg/kg (Table 1) but not with a single dose of 200 ng/kg. These data suggest that total doses in the low nanogram per kilogram body weight range may not affect reproduction in monkeys. Thus far, a no-effect level on reproductive outcomes in the rhesus monkey has not been reported, nor have any long-term (several years) or multigeneration studies been conducted in this species.

Immunotoxicity

Thigpen et al. (1975) treated 4-wk-old specific-pathogen-free male C57BL/6Jfh mice once weekly for 4 wk with 0.5, 1, 5, 10, or 20 μg TCDD/kg b.w. (equivalent to 0.07, 0.14, 0.71, 1.43, or 2.86 μg TCDD/kg

TABLE 1. Fetal Loss in Rhesus Macaques after Oral Doses of TCDD during Weeks 4 through 6 of Pregnancy[a]

Group	Total dose (μg/kg)	Schedule	Fetal losses	Gestational age of lost fetus (d)	Maternal toxicity
I	5	9 Divided doses, 3 times a week	2/2	47, 50	2/2
	1	Same	3/4	50, 57, ?	1/4
	0.2	Same	1/4	?	0/4
II	1	1 Dose, d 25	3/3	48, ?, ?	2/3
	1	1 Dose, d 30	3/3	50, 51, 55	3/3
	1	1 Dose, d 35	2/3	53, 108	1/3
	1	1 Dose, d 40	2/3	100, 100	0/3
III	0	9 Divided doses, 3 times a week	3/12	118, ?, ?	0/12

[a]Selected from McNulty (1982).

b.w.·d). A significant decrease in body weight gain was observed in the group with 2.86 μg TCDD/kg b.w.·d. A dose of 0.14 TCDD/kg b.w.· or greater for 4 wk, followed by infection with *Salmonella berne*, significantly increased mortality and decreased the time from infection to death. TCDD treatment did not affect the mortality of the mice caused by *Herpes virus suis* infection. Thymic atrophy and liver pathology were present at 2.86 and 1.43 μg TCDD/kg b.w.·d. but not at lower doses. These results are difficult to interpret; furthermore, the mice may have had preexisting immunity to the herpes virus.

In contrast, Vos et al. (1978) and Thomas and Hinsdill (1979) did not observe any impairment in the response of TCDD-treated mice to challenges with *Listeria monocytogenes*. Male Swiss mice were given 50 μg TCDD/kg b.w.·wk for 4 wk. The mice were then challenged with *Listeria*, and viable *Listeria* were measured in the spleen 2 d later. TCDD treatment did not affect spleen *Listeria* counts, nor did it impair the ability of peritoneal macrophages to reduce nitro-blue tetrazolium to formazan. Thus, TCDD treatment did not seem to affect macrophage function in mice.

Vos et al. (1978) studied the susceptibility of TCDD-treated mice to *Escherichia coli* endotoxin. Three- to 4-wk-old specific pathogen-free Swiss mice were dosed by gavage once a week for 4 wk with 0, 1.5, 5, 15, or 50 μg TCDD/kg b.w. (equivalent to 0, 0.21, 0.71, 2.14, or 7.14 μg TCDD/kg b.w.·d). Two days after the final dose of TCDD, the mice were injected intravenously with *E. coli* endotoxin, and mortality was assessed after 48 h. A TCDD dose-related increase in sensitivity to *E. coli* endotoxin was observed. With 10 μg endotoxin, the no-effect dose was 0.71 μg TCDD/kg b.w.·d over 4 wk.

Thomas and Hinsdill (1979) studied 5- to 6-wk-old mice from specific-pathogen-free mothers fed diets containing 1, 2.5, or 5 μg TCDD/kg. The offspring were injected with a range of doses of *Salmonella typhimurium* lipopolysaccharide endotoxin, and mortality was assessed over 14 d. A TCDD dose-related increase in mortality was observed with a no-effect level of 1.0 μg TCDD/kg diet (0.15 μg/kg b.w.·d) when 40 μg endotoxin was administered.

Vos and Moore (1974) assessed cell-mediated immune functions in rats exposed to TCDD prenatally or prenatally and postnatally. Fisher-344 rats received 0, 1, or 5 μg TCDD/kg b.w. on d 11 and 18 of gestation. In some cases, the pups remained with treated mothers during lactation, whereas other groups suckled nontreated mothers. The transformation of spleen cells by the phytomitogen phytohemagglutinin on a per cell basis was significantly reduced in rats postnatally exposed to TCDD. The response of spleen cells to phytohemagglutinin on a per spleen basis was reduced to 72% of that of controls in rats from dams dosed with 1 μg TCDD/kg b.w. on d 11 and 18 of gestation. The response of thymus cells to phyto-hemagglutinin (on a per cell basis) was significantly reduced in rats from dams given 5 μg TCDD/kg b.w., and the response to concanavalin A was significantly decreased on a per thymus basis.

Graft-versus-host reactions and allograft rejection have also been studied. Vos and Moore (1974) reported prolonged allograft rejection times in rats and reduced graft-versus-host reactions in rats and mice after exposure to TCDD. The rats were exposed to TCDD through the dam's milk. The offspring of dams that were dosed with 5 μg TCDD/kg b.w. on d 11 and 18 of gestation and on d 4, 11, and 18 of lactation showed significant effects in both the allograft rejection time and graft-versus-host reactions, whereas lower dosages did not cause any observable effects. Mice treated with 0.14, 0.71, and 3.57 μg TCDD/kg b.w.·d showed a decrease in graft-versus-host reactivity.

In addition, Faith et al. (1978) and Faith and Luster (1979) reported that TCDD suppressed delayed hypersensitivity in Charles River albino rats given 5 μg TCDD/kg b.w. on d 18 of gestation, then 5 μg TCDD/kg b.w.· wk. Some pups were cross-fostered onto untreated dams to provide a prenatal exposure group. Oxazolone was used as the contact sensitizing agent, and the radiometric ear assay also was used. A suppression in the delayed hypersensitivity reaction was observed in all exposure groups.

Thomas and Hinsdill (1979), using dinitrofluorobenzene as the sensi-tizing agent, studied the effects of TCDD exposure on delayed hypersensi-tivity reactions by measuring the increase in ear thickness. Swiss Webster mice were fed diets containing 0, 1, 2.5, and 5.0 μg TCDD/kg. A significant suppression in the delayed hypersensitivity reaction was observed in offspring from dams on the 5-μg/kg diet (about 0.75 μg TCDD/kg b.w.·d) tested at 5 wk of age.

In summary, some studies suggest that the humoral immune function may be affected in rodents (Vos and Moore, 1974; Thomas and Hinsdill,

001282

1979; Vecchi et al., 1980), although Faith et al. (1978) and Faith and Luster (1979) were not able to substantiate these findings. TCDD effects on the cellular immune function have been more consistently shown, although no chronic studies have been conducted to more precisely characterize this effect.

The studies done in rats and mice suggest that the developing immune system is more susceptible to the effects of TCDD than the adult immune system. On the other hand, in the guinea pig the thymus is the primary target organ for TCDD, and weekly doses of 40 ng TCDD/kg b.w. for 8 wk depressed the delayed hypersensitivity reaction to tuberculin (Vos et al., 1973) in adult guinea pigs.

Several other studies have been conducted in guinea pigs, rats, and mice to evaluate the immune response, but they do not give any additional information about dose response. Although the immunotoxicity of TCDD is a serious health effect in animals—apparently present at low doses of TCDD exposure—we cannot use these data in risk analysis at this point, since no adequate dose-response data exist.

Carcinogenicity and Other Chronic Toxic Effects

A study by Van Miller et al. (1977) in Sprague-Dawley rats suggested that TCDD is carcinogenic. However, only 10 rats per group were used. The results of that study are summarized in Table 2. It is surprising that no tumors at all were observed among the controls. Since there was no increase of any particular tumor but only an increase of total tumors, these data are not very useful. The findings were not statistically significant, and the number of animals tested was small.

Subsequently, Kociba et al. (1978) reported a study in which groups of 50 male and 50 female Sprague-Dawley rats (Spartan substrain) were fed diets containing 22, 208, and 2193 ng TCDD/kg for 2 yr. This is equivalent to daily doses of 0.001, 0.01, and 0.1 μg TCDD/kg b.w. The total intake of TCDD for rats surviving 24 mo would be 0.73, 7.3, and 73 μg TCDD/kg b.w. for the 3 treated groups. The controls consisted of 86 male and 86 female rats.

Numerous toxicologic effects were observed at 0.1 μg TCDD/kg b.w.·d. These effects included increased mortality; decreased body weight gain; depressed hematological parameters; increased urine levels of porphyrins and δ-aminolevulinic acid; increased serum enzyme activity for alkaline phosphatase, γ-glutamyl transferase, and glutamic-pyruvic acid transaminase; and morphological changes in hepatic, lymphoid, respiratory, and vascular tissues.

At 0.01 μg TCDD/kg b.w.·d, liver toxicity, focal pulmonary alveolar hyperplasia, and, in females, increased urinary porphyrin excretion were noted. No toxic effects of significance were reported in rats exposed to 0.001 μg TCDD/kg b.w.·d for 24 mo. A summary of the tumor incidence observed by Kociba et al. (1978), for tumors whose incidence in treated

TABLE 2. Summary of Neoplastic Alterations Observed in Sprague-Dawley Rats Fed Subacute Levels of TCDD for 78 Weeks[a]

Level of TCDD (ng/kg)	Number of animals with neoplasms[b]	Number of neoplasms	Diagnosis
0	0	0	—
1	0	0	—
5	5	6	1 Ear-duct carcinoma 1 Lymphocytic leukemia 1 Adenocarcinoma (kidney) 1 Malignant histiocytoma (peritoneal)[a] 1 Angiosarcoma (skin) 1 Leydig-cell adenoma
50	3	3	1 Fibrosarcoma (muscle) 1 Squamous-cell tumor (skin) 1 Astrocytoma (brain)
500	4	4	1 Fibrosarcoma (muscle) 1 Carcinoma (skin) 1 Adenocarcinoma (liver) 1 Sclerosing seminoma (testes)
1000	4	5	1 Cholangiocarcinoma (liver) 1 Angiosarcoma (skin) 1 Glioblastoma (brain) 2 Malignant histocytomas (peritoneal)[c]
5000	7	10	4 Squamous-cell tumors (lung) 4 Neoplastic nodules (liver) 2 Cholangiocarcinomas (liver)

[a]Van Miller et al. (1977).
[b]10 animals per group.
[c]Metastases observed.

and control rats was significantly different, is presented in Table 3.
    Toth et al. (1979) conducted studies in Swiss/H/Riop mice. Three groups of 45 male mice were given weekly doses (by gavage) of 7.0, 0.7, or 0.007 μg TCDD/kg b.w. for 1 yr, then studied for their entire lifetimes. An equal number of control mice (45) were given the TCDD vehicle (sunflower oil) each week. The incidence of liver tumors was significantly increased in the 0.7 μg TCDD/kg b.w.·wk group (48% tumor incidence),

TABLE 3. Total and Individual Tumors in Treated Male and Female Rats of Significantly Different Incidence than Those in Nontreated Control Rats[a]

Tumor or tumor-like lesion	Dose level (µg TCDD/kg b.w.·d)							
	0		0.001		0.01		0.1	
	M (n = 85)[b]	F (n = 86)	M (n = 50)	F (n = 50)	M (n = 50)	F (n = 50)	M (n = 50)	F (n = 49)
Hepatocellular neoplastic nodules	6	8	0	3	3	18[c]	2	23[c]
Hepatocellular carcinoma	2	1	0	0	3	2	1	11[c]
Stratified squamous-cell carcinoma of hard palate or nasal turbinates	0	0	0	0	0	1	7[c]	4[c]
Keratinizing squamous-cell carcinoma of lung	0	0	0	0	0	0	1	7[c]
Benign tumor of uterus	—	28	—	12	—	11	—	7[d]
Benign neoplasm of mammary gland	—	73	—	35	—	36	—	24[d]
Mammary carcinoma	—	8	—	4	—	4	0	0[d]
Pituitary adenoma	26	43	6	18	11	13	13	12[d]
Subcutaneous carcinomas	10	1	1[d]	1	5	0	6	0
Acinar adenoma of pancreas	14	0	7	1	5	0	2[d]	1
Adenoma of adrenal cortex	0	9	0	6	2	2	5[c]	5
Pheochromocytoma of adrenal gland	28	7	6	3	10	1	4[c]	2

[a]Kociba et al. (1978).
[b]Number of rats examined = n.
[c]Statistically greater than control data when analyzed by using the Fischer exact probability test, $p < 0.05$.
[d]Statistically less than control data when analyzed by using the Fischer exact probability test, $p < 0.05$.

63

001284

compared with that of the control group (18% tumor incidence). The incidence of liver tumors observed in the 7.0 µg TCDD/kg b.w.·wk group was 30% greater but statistically was not significantly different from that of the control group. Increased mortality in this group may account for not finding a statistical significance.

The National Toxicology Program (NTP) (1982a,b) also conducted carcinogenicity studies in rats and mice. TCDD was investigated in groups of 50 male and 50 female Osborne-Mendel rats and 50 male B6C3F1 mice (0.01, 0.05, or 0.5 µg/kg·wk) and in 50 female mice (0.04, 0.2, or 2.0 µg/kg·wk). TCDD was suspended in a vehicle of 9:1 corn oil:acetone and administered by gavage 2 X/wk for 104. wk. A dose-related depression in mean body weight gain was observed in male and female rats, compared with groups of 75 vehicle controls. Significant increases were observed in incidences of follicular-cell adenomas in the thyroid in male rats, neoplastic nodules of the liver in high-dose female rats, hepatocellular carcinomas in male and female mice, follicular adenomas in the thyroid in female mice, and toxic hepatitis related to TCDD administration in high-dose rats and mice of both sexes. Under the conditions of this bioassay, TCDD was carcinogenic for both Osborne-Mendel rats and B6C3F1 mice (Table 4). In addition, the carcinogenicity of an acetone suspension of TCDD applied to the clipped backs of 30 male and 30 female Swiss-Webster mice 3 X/wk for 99 or 104 wk was investigated. Females received 0.005 µg TCDD/application, and males, 0.001 µg TCDD. Vehicle controls consisted of 45 mice of each sex treated with 0.1 ml acetone, 3 X/wk. Mean body weights of dosed male and vehicle-control mice were less than those of untreated male controls throughout the study; for females, mean body weights were less than those of untreated controls for the first 80 wk. An increased incidence of pyelonephritis was observed in male mice exposed to acetone alone or in combination with TCDD. A statistically significant increase in the incidence of fibrosarcoma of the integumentary tissue was observed in female mice given TCDD and TCDD after DMBA, compared with controls. Fibrosarcomas appeared significantly earlier in TCDD-dosed males than in vehicle controls, although the increased incidence of such tumors was not statistically significant.

In females, the incidence of fibrosarcoma in the integumentary system was 30% (8/27 mice) in the treated group and 5% (2/41 mice) in the controls. In the males, the incidence of the same tumor type was not significantly different—21% (6/28) among the exposed mice and 7% (3/42) among the controls.

If the cancer studies conducted by Kociba et al. (1978) and by the NTP (1982a,b) are compared, it is evident that the tumors in the liver are produced at quite similar dosage levels. (See also "Considerations for Risk Assessment.") Although increased incidence of tumors in other organs was observed by the NTP and by Kociba et al., the target organs varied in the

TABLE 4. Incidence of Dose-Related Tumors in Osborne-Mendel Rats and B6C3F1 Mice Treated with TCDD[a]

Tumor type	Dose (μg/kg·wk)	Sex	Incidence
Osborne-Mendel rats			
Follicular-cell	0	M	1/69
adenomas, thyroid	0.01	M	5/48
	0.05	M	6/50
	0.5	M	10/50 ($p = 0.001$)
	0	F	3/73
	0.01	F	2/45
	0.05	F	1/49
	0.5	F	6/47
Neoplastic nodules	0	F	5/75
of the liver	0.01	F	1/49
	0.05	F	3/50
	0.5	F	12/49 ($p = 0.006$)
Neoplastic nodules	0	F	5/75
or hepatocellular carcinoma	0.01	F	1/49
	0.05	F	3/50
	0.5	F	14/49 ($p = 0.001$)
B6C3F1 mice			
Follicular-cell	0	F	0/69
adenomas, thyroid	0.04	F	3/50
	0.2	F	1/47
	2.0	F	5/46 ($p = 0.009$)
Hepatocellular carcinoma	0	M	8/73
	0.01	M	9/49
	0.05	M	8/49
	0.5	M	17/50 ($p = 0.002$)
	0	F	1/73
	0.04	F	2/50
	0.2	F	2/48
	2.0	F	6/47 ($p = 0.014$)
Osborne-Mendel rats			
Lymphoma and leukemia	0	F	18/74
	0.04	F	12/50
	0.2	F	13/48
	2.0	F	20/47

[a]National Toxicology Program (1982a, b).

two studies. This may be caused, in part, by differences in dosing (gavage versus exposure to TCDD in ground feed) and differences in the strains of rats used.

At the CDC consultants' meeting, in written comments by one of the consultants, and at a meeting in Cincinnati where the EPA criteria documents on TCDD in water and air were reviewed (EPA Dioxin Meeting, July 27–29, 1983), it was pointed out that the carcinomas of the lungs and upper respiratory tract in the study by Kociba et al. (1978) could conceivably have been caused by direct contact of TCDD-contaminated feed particles with the respiratory tract. The reasons for this are as follows.

In the Kociba study, TCDD was offered to the rats in ground feed. Rats exposed to ground food often have food particles in their airways and lungs, and this is noted on microscopic examination. In the NTP (1982b) study, TCDD was given by gavage, and liver tumors, but not tumors of the respiratory tract, developed. The direct dose to the respiratory tract cannot be estimated in the rats that developed cancer of the respiratory tract. In localized areas, it most probably was higher than the total daily dose calculated on a body-weight basis.

PROMOTER VERSUS INITIATOR

Although the available evidence shows that TCDD has a tumor-promoting capacity, there is, as yet, very little to suggest that it is also an initiator.

Poland et al. (1982) recently presented evidence of TCDD tumor promotion in skin in HRS/j hairless mice, and Pitot et al. (1980) showed TCDD to be a potent tumor promoter in a two-stage model of carcinogenesis in rat liver. The amount of TCDD bound to DNA is 4 to 6 orders of magnitude less than that for other known carcinogens (Poland and Glover, 1979). However, it has not been established what possible role any metabolites of TCDD may play. Binding to DNA does not necessarily mean that the DNA is altered, nor is it possible to determine with absolute certainty that TCDD has no initiating properties.

HUMAN HEALTH EFFECTS

Most of our information about the human health effects of TCDD has been obtained from studies of workers who were exposed to TCDD during the production or handling of 2,4,5-trichlorophenol and products made from this chemical; as noted previously, precise exposure data, necessary for dose-response calculations, are not available for these situations. In some plants, workers primarily developed chloracne but no systemic illness (May, 1982). Other authors have reported complaints of weight loss, easy fatigability, aching muscles, insomnia, irritability, loss of libido, and

sensory changes. The liver may become tender and enlarged, and decreases in nerve conduction velocity have been reported. Total serum lipids may be increased, and the prothrombin times may be prolonged (IARC, 1977; Bauer et al., 1961; Bleiberg et al., 1964; Jensen and Walker, 1972; Oliver, 1975). Porphyria cutanea tarda has also been observed (Jirasek et al., 1976; Bleiberg et al., 1964; Poland et al., 1971).

Follow-up studies in exposed workers have not been very informative, partly because the number of workers included has been small. The largest of these groups consisted of 121 workers (Zack and Suskind, 1980).

May (1982) reported that 10 years after an incident in which 79 workers developed chloracne because of exposure to TCDD, half of the affected subjects still had chloracne. No other adverse effects were reported.

In another episode, reported by Pazderova-Vejlupkova et al. (1981), the condition of many patients with relatively severe early findings improved over the years. Apparently, during 1965 to 1968, 80 workers who had been engaged in the production of 2,4,5-sodium trichloro-phenoxyacetate and the butyl ester of trichlorophenoxyacetic acid became ill after TCDD exposure. A 10-yr follow-up study was conducted of 55 exposed individuals. Most of the patients had initially developed chloracne, and 11 manifested porphyria cutanea tarda. About half of the patients had elevated lipids with abnormalities in the lipoprotein spectrum; two-fifths had abnormal glucose tolerance tests; one-third had elevated liver function tests; and the liver tissues from liver biopsy material of selected patients fluoresced under ultraviolet light, indicating elevated prophyrins. Most suffered from various psychological disorders. As of this date, two patients have died of bronchogenic lung carcinoma; one of liver cirrhosis; one of a rapidly developed, extremely unusual type of atherosclerosis precipue cerebri; and two patients have died from traffic injuries. No conclusions can be drawn from this list of fatalities, since these are conditions that normally occur in the general population and since there were only very few deaths. The conditions of most other patients have improved.

Hardell and Sandstrom (1979) and Eriksson et al. (1981) conducted two case-control studies in Sweden and reported an increased risk of soft-tissue sarcomas in men who were exposed either to trichlorophenols or to phenoxy herbicides during their application. These authors also reported a third case-control study from Sweden that suggests that phenoxy acids and chlorophenols may also predispose to Hodgkin's lymphoma, but as yet there is little support for this theory from other sources (Hardell et al., 1981). The Swedish studies were recently summarized and discussed by Coggon and Acheson (1982), who concluded that "further research is urgently needed to confirm or refute these associations, to define the extent of the risk (if any) and to identify the carcinogen(s)."

The Swedish results could not be substantiated by Milham (1982). Preliminary results from a case-control study under way in New Zealand

also have not indicated an excess risk of soft-tissue sarcoma (Smith et al., 1976, 1983). The mortality rate for soft-tissue sarcomas for United States males between 40 and 64 yr of age ranges from about 5 to 20 per 1,000,000.[1] This low incidence severely limits the power in some studies to detect such rare events. The Swedish studies, however, are supported by results from the United States (Honchar and Halperin, 1981; Cook, 1981) in workers producing these chemicals. In the United States four follow-up studies were conducted among workers exposed to 2,4,5-trichlorophenol or 2,4,5-T (Cook et al., 1980; Ott et al., 1980; Zack and Gaffney, 1983; Zack and Suskind, 1980). All of these investigators concluded that there were no excess deaths due to any cause. However, each of three cohorts had one death due to soft-tissue sarcoma. Honchar and Halperin (1981) reviewed the four studies and noted that in the four merged cohorts there were a total of 105 deaths, 3 of which (2.9%) were due to soft-tissue sarcoma. On the basis of national rates of death for men aged 20 to 80, only 0.07% of deaths due to soft-tissue sarcoma would have been expected. Recently, another person in one of the four cohorts died because of a soft-tissue sarcoma (Cook, 1981; M. G. Ott, personal communication), brininging the total to four deaths due to soft-tissue sarcoma in the four merged U.S. cohorts. Microscopic review of tissue sections from these tumors and three additional cases was recently done. Two of the 4 cases with documented evidence of exposure and 3 additional cases that did not have documented evidence of exposure were confirmed to represent soft-tissue sarcoma (M. A. Fingerhut and co-workers, 1984, National Institute for Occupational Safety and Health, Cincinnati, Ohio 45226; manuscript in preparation). The question has also been raised as to whether it is appropriate to merge these separate cohorts.

Thiess et al. (1982) reexamined a cohort of 74 persons who had been exposed to dioxin 27 yr earlier during an uncontrolled reaction at a trichlorophenol production facility. Overall mortality (21 deaths) did not differ in this group from the rate expected in 3 external reference populations or from that observed in 2 internal comparison groups. Of the 21 deceased persons, 7 had cancer (ICD No. 140-199), compared with an expected 4.1 ($p = 0.14$). Three deaths due to stomach cancer (ICD No. 151), at ages 64, 66, and 69 yr, were found, compared with 0.6 expected ($p = 0.024$) from regional mortality data. One stomach cancer occurred among 148 individuals in the two comparison cohorts.

Despite the increasing number of reports suggesting a positive association of soft-tissue sarcomas and exposure to chemicals known to be contaminated with chlorinated dibenzodioxins, several of the CDC consultants expressed caution or skepticism. For example:

[1] Because of the difficulty in properly identifying soft-tissue sarcomas microscopically, the background incidence of this tumor in the general population is not well defined.

Epidemiological reports have included prospective cohort studies (some with more than 3000 person-years) in which exposures have been well documented, even if not well quantified, in which no excess mortality or malignancies were observed. The few case-control reports that suggest an excess risk of cancer are compromised by their poorly documented and obviously heterogeneous exposures, uncertainty that controls were appropriately selected, and the potential for introduction of a serious error through recall bias. Additionally, one of the studies mentioned in the review by Coggon and Acheson deals with phenoxy-acetic acid herbicides known not to be contaminated with TCDD.

The possibility of a relationship between TCDD exposure and various soft tissue neoplasms, mesenchymal tumors, and sarcomas has been raised. Grouping of these diverse tumor types is not appropriate. However, the question of a possible causal relationship of TCDD and soft tissue tumors needs to be explored further. [Note: Since these tumors are extremely rare the negative studies done to date may have insufficient power to detect them.]

Another consultant added:

A number of case-control and follow-up studies have been conducted in worker populations. Each suffers from one or more deficiencies: (1) a lack of sufficient measured exposure, (2) a lack of sufficient time to develop disease between the exposure and the study, (3) a population size too small to reasonably find cases of soft tissue sarcoma, or (4) possible lack of contamination of the commercial product with dioxin.

These comments illustrate some of the present controversies. It is hoped that larger epidemiologic studies, such as the study based on a follow-up of workers in the National Institute for Occupational Safety and Health (NIOSH) dioxin registry, will resolve these issues.

Useful information from studies of health effects following environmental exposure is sparse (Pocchiari et al., 1979). After an explosion at the ICMESA plant in 1976 (Hay, 1976), children in Seveso developed chloracne (Reggiani, 1980). Results of some liver-function tests were elevated in that population (Reggiani, 1980), and the incidence of abnormal results of nerve conduction tests was reported to be statistically significantly elevated in subjects with chloracne (Fillipini et al., 1981). A child in Missouri (Carter et al., 1975) who played in dirt contaminated with 30 ppm TCDD in some areas of a riding arena had hemorrhagic cystitis. Claims have been made that exposure to 2,4,5-T contaminated with TCDD has resulted in an increased incidence of spontaneous abortions, malformations, cancer, and other health problems (Milby et al.,

1980; Consultative Council on Cogenital Abnormalities, 1978). Since the studies reporting such results have severe methodologic limitations, additional well-designed studies need to be conducted before any conclusions can be drawn about these health effects in the general population.

One problem with all of the human studies, including reports from workers, is that direct objective measurement of exposure is not available. In situations where no systemic health effects were observed, absorption of TCDD may have been minimal or nonexistent. For instance, the highest soil level in Zone A in Seveso close to the factory was 55 ppb ($\mu g/kg$), whereas levels on the vegetation ranged from nondetected to 15.8 ppm. Of the 44 vegetation samples analyzed, 33 had less than 1 ppm TCDD. Most of the vegetation was removed early, and people in the area closest to the factory were evacuated 2 wk after the event and were warned not to eat vegetables from their gardens. The area where people are living now (Zone B) has soil levels below 0.15 ppb. Although several comments were received that exposure in Seveso was substantial, soil contamination levels in Missouri are 10–1000 times higher than in Seveso. For additional information on environmental contamination in different parts of the world, the reader is referred to Reggiani (1980).

CONSIDERATIONS FOR RISK ASSESSMENT

The following considerations and calculations were made for soil contaminated with TCDD in two residential areas in Missouri.

Exposure Assessment at TCDD-Contaminated Areas

Questions as to the habitability of any area where soil is contaminated with TCDD are necessarily linked to considerations of excess risks of developing specific adverse health effects as a result of the total cumulative dose an individual receives. In turn, this cumulative dose is a function of several factors:

1. Concentrations of environmental contamination
2. Location of and access to contaminated areas
3. Type of activities in contaminated areas
4. Duration of exposure
5. Specific exposure mechanisms

Dose-rate may be an important factor in exposure assessment, but its effect on carcinogenic risk for dioxin is uncertain. The potential of increased risk from receiving high doses at susceptible life stages may be balanced or exceeded by repair mechanisms operative at periods of lesser dose. This cannot be ascertained based on currently available information. We have therefore based the exposure calculations in this section on cumulative dose averaged over a lifetime.

In addition, questions of continued habitability must also include considerations of the potential for limiting or eliminating ongoing exposures.

Developing a level of concern about an unacceptable risk due to exposure to TCDD poses significant difficulties because TCDD has such unique properties, as outlined in the preceding pages of this paper.

In the past, as a first approach, several groups have used a series of risk-assessment estimates based on several of these factors to determine what an "acceptable" risk for exposure to TCDD would be. As more information on the toxicity of this chemical has become available, these levels have generally been reduced.

To determine whether a specific concentration of TCDD in soil presents a risk to humans, we must first examine how humans might absorb TCDD from such soil. Unfortunately, the amount of any chemical present in soil that may be absorbed by humans coming in contact with the soil is not well known. Most risk assessments that have been made in the past have been made for such media as food, where it is assumed that a certain amount of food with a certain concentration of the chemical in it is consumed; for air, where how much air is inhaled simply needs to be calculated; or for chemicals in water, where the only number needed is the amount of water consumed—although, as far as water-quality criteria are concerned, the bioaccumulation of chemicals in fish from contaminated water is also considered. Unfortunately, the analogous series of estimates is more complicated for soil.

Basically, three exposure routes must be considered: dermal absorption through direct contact with the soil, ingestion of soil, and the inhalation of dust to which TCDD is attached. Vapors may be an additional, probably minor, route of exposure. Another issue, which does not directly enter into the current risk assessment, is the fact that TCDD in the environment could eventually end up in the food chain, particularly in fish. If TCDD enters a food chain, there is an unknown additional source of exposure which must be added to the risk of those individuals exposed to contaminated soil and of a larger, undefined population.

Regarding dermal absorption, there is some evidence that TCDD binds to the soil and would not be as easily available for absorption. (Vegetation covering contaminated soil may also decrease TCDD availability.). Information on bioavailability, however, is currently limited and may vary for different types of soil. According to the literature (Poiger and Schlatter, 1980, and a personal communication), anywhere from 1 to 10% of the TCDD in the soil may be absorbed through the skin, and this percentage is likely to depend on the TCDD concentration in the soil (i.e., it may be greater at higher concentrations) and on the type of soil. When Poiger and Schlatter (1980) applied soil with a dose of 350 ng TCDD to the backs of rats, $1.7 \pm 0.5\%$ of the dose was found in the liver; at a dose of 26 ng, about 0.05% of the dose was found in the liver. The authors do not state

in the article how long the soil was left in contact with the skin of the rats,[1] except that after 24 h it was not possible to recover all of the applied dose from the skin surface. Therefore, the subsequent estimates will allow for this range of skin absorption factors.

In regard to the portion of total dose due to ingestion of soil particles, feeding studies in animals suggest that 30% or more of the TCDD adsorbed on soil will be absorbed in the gastrointestinal tract (E. E. McConnell et al., 1984). Poiger and Schlatter (1980) found 16–24% of the administered dose of TCDD in the liver. According to Fries and Marrow (1975), this represents about 70% of the body burden of TCDD.

In regard to inhaled doses, little information is available on the amount of dust that may be present in the air in situations of known soil contamination; measurements in Seveso showed that the amount of dust in air was 0.14 mg/m³ air (DiDomenico et al., 1980). No dust levels in air whose sole source is soil are available from air monitoring stations. Soil, vegetable matter, and particles from other sources such as car exhaust are measured as particulate matter. The use of particulate matter would highly overestimate dust derived from soil. In riding arenas or in relatively drier areas, dust levels would be possibly higher.

On the other hand, immediately after a rainfall there would probably be less dust. In the same investigation, it was shown that TCDD levels in dust were comparable to those found in soil. Another unknown is the amount of material that could be carried into the house from the outside. It is conservatively assumed that the exposure to dust inside a house surrounded by contaminated soil is similar to the exposure that would occur if people spent their entire time in contact with the contaminated soil outside. (One of the CDC consultants commented that the assumption that indoor levels will equal outdoor levels appears unnecessarily conservative.) An average adult at rest exchanges approximately 10 m³ air/24-h-period, and this would increase with mild activity to 18–24 m³/d, and to 40 m³/d with hard physical labor. Finally, it is assumed that whatever TCDD is inhaled adsorbed to dust particles is absorbed either through deposition in the respiratory tract or by ingestion after being brought up by the ciliary action of the respiratory tract epithelial cells.

Several comments were received from CDC consultants on exposure estimates: e.g., how much soil does a young child eat when playing outside? how much soil gets onto the skin during gardening activities? how much soil gets onto the skin of children playing football or other games? Unfortunately, there is no documentation in the literature that clarifies the problems raised. For illustrative purposes, 1 g soil less than 1 mm thick can be spread over an area of 4–5 cm² or 1½–2 in². Ten grams of soil less than 1 mm thick can be spread over an area of about 15 cm² or about 6 in². (The volume of dirt will vary somewhat with moisture content.) The soil used in calculating the above surface areas was

[1] 24 h. (Poiger, 1984, personal communication.)

Georgia clay that had been stored for several months at room temperature.

All of the calculations regarding exposure are based on the assumption that humans have access to and contact with the contaminated soil and that a percentage of the TCDD present in the soil is absorbed. The frequency of access and contact must also be considered, and for dermal exposure it must be remembered that clothing will afford some protection. The doses calculated below are, in some sense, worst-case estimates for the concentrations used.

A large number of estimated total daily doses can be derived from the many combinations of the exposure route-specific doses (given different sets of assumptions as to absorption rates, soil contamination, etc.). However, in an attempt to derive a more accurate estimate of exposure to and uptake from contaminated soils, a simulation analysis was performed utilizing a further set of assumptions, which are most likly to be obtained in reality. For instance, TCDD is suggested to have an environmental half-life of approximately 10–12 yr. Therefore, the simulation model assumed that soil contamination levels would decrease in a log-linear fashion with a 12-yr half-life following the equation

$$TCDD_t = e^{(a - 0.00016t)}$$

where $TCDD_t$ = current soil level on day t (ppb)
a = ln (initial soil contamination level, ppb)
t = number of days elapsed since initial soil contamination measurement

Consideration of the route-specific uptakes does not require an assumption of constant exposure but merely postulates an a priori reasoned absolute daily amount of soil contact. For inhalation, we assumed that airborne dust is contaminated at the same level as surrounding outside soil and that 15 m^3 air is exchanged per day. In regards to ingestion, based on preliminary results of bioavailability studies, a GI absorption rate of 30% from soil seems most tenable. In addition, the amount of soil that the average person is likely to ingest will be dependent on characteristic activity patterns, which, in turn, are closely dependent on age. Based on work done studying lead uptake from contaminated soils, a reasonable pattern of soil ingestion (which still maintains conservative estimates) for specific age groups was constructed and is presented in Table 5. Based on animal experimentation, the best estimate for dermal absorption of TCDD is approximately 1% (especially in the low ppb contamination ranges). Reasoning for an age-dependent pattern of deposition of soil on exposed skin similar to soil ingestion was used to derive Table 6. Finally, it was assumed that these exposures would be likely to take place only 6 mo of the year because of seasonal influences and varying activity patterns.

The simulation model was run using a computerized iterative procedure by daily increments to estimate total lifetime dose using the following formula:

$$\text{Total lifetime dose} = \sum_{t=1}^{T} \text{TCDD}_t \ (\text{ING}_t \times \text{GI} + \text{DERM}_t \times \text{ABS} + \text{INH} \times \text{DUST}) \ \text{SEAS}$$

where T = expected lifespan (d)
  ING_t = age-specific amount of soil ingested at time t
  GI = % absorbed through gastrointestinal tract
  DERM_t = age-specific amount of soil deposited on skin at time t
  ABS = % absorbed through skin
  INH = amount of air exchanged per day
  DUST = concentration of dust in air
  SEAS = "dummy" variable for seasonal access to outdoor con-
taminated areas (i.e., =1 for fair-weather months and 0 for
cold weather months)

For the sake of brevity, extreme total daily dose estimates were compiled for two divergent levels of TCDD soil contamination (1 ppb and 100 ppb). Based on the assumptions set forth above, at an initial soil contamination of 1 ppb in residential areas, the average daily TCDD dose to an individual over a 70-yr lifetime would be 44.6 pg. This is equivalent to 636.5 fg/kg b.w.·d for a 70-kg person. For soils initially contaminated at 100 ppb, the average daily dose is estimated at 4.5 ng or 63.7 pg/kg b.w. for a 70-kg individual (Fig. 1).

In addition, it must be recognized that not all soil is contaminated uniformly in a given area. At the 1-ppb level the dose (and the resulting risk) would be considerably reduced if only 1% or 10% of the accessible soil contained TCDD at concentrations of 1 ppb and the rest of the soil were not contaminated (illustrated in Fig. 1).

Risk Assessment

The critical step in assessing individual risks at these estimated dose levels must incorporate a comparison to known (or estimated) "safe" levels of exposure in relation to clearly defined health effects end points.

The National Research Council of Canada (NRCC) has recently published a report reviewing available toxicity data for TCDD and related compounds and various procedures for calculating a "virtually safe dose"

TABLE 5. Estimated Daily Soil Ingestion Patterns by Age	
Age group	Soil ingested
0–9 mo	0 g
9–18 mo	1 g
1½–3½ yr	10 g
3½–5 yr	1 g
5 yr	100 mg

TABLE 6. Estimated Daily Deposition of Soil on Skin by Age	
Age group	Amount of skin
0–9 mo	0 g
9–18 mo	1 g
1½–3½ yr	10 g
3½–15 yr	1 g
15 yr	100 mg

(VSD) for TCDD from such data. A summary table from this document listed the various models, estimated VSDs, approximate 95% confidence levels, and references to the different models that were employed. This table has two typographical errors. The virtually safe dose differs from other calculations because only liver-tumor data and body weight instead of body surface were used. The analyses in this section follow guidelines recommended by the CDC consultants and are based upon two studies: one by Kociba et al. (1978), and another by the National Toxicology Program (NTP, 1982b). Further details on these (and other) studies can be obtained from the EPA TCDD risk assessment (EPA number: EPA-600/6-81-003). All of the CDC consultants agreed that the available human data are inadequate to be used in risk assessment calculations.

In the Kociba study, a substantial proportion of the animals, including those in the control group, died before the 2-yr sacrifice (78–92% in the males and 68–92% in the females). In addition, there appeared to be time-related and dose-related effects at the lesion sites. Results from a time-adjusted analysis of these data did not differ markedly from results reported in this document (C. Portier et al., 1984).

The important lesion sites (for risk assessment) in the two sexes are given in Table 7, along with the tumor incidence at each dose. The original pathology done by Kociba was reviewed by Squire, and the results of Squire's review (U.S. EPA 1980a) are therefore also included. In the EPA's risk assessment, the agency based its analysis upon grouping these sites, using as incidence the number of animals with any one of the lesions divided by the number of animals examined at any of these sites for each sex. We have not used this procedure; instead, the multistage model (Crump et al., 1977) was fitted to the tumor incidence from each lesion site. These results appear in Table 8, along with the chi-squared value for

TABLE 7. Important Lesion Sites in the Kociba TCDD Study

| Site | Sex | Pathologist | Control | TCDD dose (μg/kg b.w.·d) | | |
				0.001	0.01	0.1
Stratified squamous-cell carcinoma of the tongue	Males	Kociba	0/76	1/49	1/49	3/42
		Squire	0/77	1/44	1/49	3/44
Nasal turbinates/hard palate squamous-cell	Males	Kociba	0/51	0/34	0/27	4/30
		Squire	0/55	1/34	0/26	6/30
	Females	Kociba	0/54	0/30	1/27	4/24
		Squire	0/54	0/30	1/27	5/22
Hepatocellular nodules and carcinoma	Females	Kociba	9/86	3/50	18/50	34/48
		Squire	16/86	8/50	27/50	33/47
Lung keratinizing squamous-cell carcinoma	Females	Kociba	0/86	0/50	0/49	7/49
		Squire	0/86	0/50	0/49	8/47

TABLE 8. Estimates and Approximate 95% Lower Confidence Limits for the VSD[a] of TCDD from the Kociba Study in Sprague-Dawley Rats

Lesion	Pathologist	Model[b]	Added risk		Chi-squared G-O-F[c]
			1/10,000	1/1,000,000	
Male rat stratified	Kociba	Linear[d]	142581 (59480)	1426 (595)	1.43
squamous-cell carcinoma	Squire	Linear[d]	151793 (62642)	1518 (626)	1.60
of the tongue					
Male rat nasal turbinates	Kociba	Cubic[d]	8877014 (17493)	1912466 (175)	0.00
or hard palate		Linear	77507 (37566)	775 (376)	0.39
squamous-cell carcinoma	Squire	Cubic[d]	7757813 (11676)	1671343 (117)	2.39
		Linear	49468 (26431)	495 (264	2.72
Female rat lung	Kociba	Cubic[d]	8659951 (32477)	1865707 (325)	0.01
keratinizing squamous-		Linear	72600 (41318)	726 (413)	0.82
cell carcinoma	Squire	Cubic[d]	8126063 (26834)	1750685 (268)	0.01
		Linear	60375 (35490)	604 (355)	0.98
Female rat nasal	Kociba	Linear[d]	49771 (25812)	498 (258)	0.49
turbinates or hard	Squire	Linear[d]	37237 (20313)	372 (203)	0.20
palate squamous-cell					
carcinoma					
Female rat liver hepato-	Kociba	Linear[d]	7742 (5725)	77 (57.2)	6.35
cellular carcinoma		Transf[e]	3836 (2919)	38 (29.1)	2.18
or adenoma	Squire	Linear[d]	8649 (6074)	86 (60.7)	10.36
		Transf[e]	3760 (2763)	38 (27.6)	4.30

[a]Entries are virtually safe dose (VSD) linear-cubic lower confidence bounds (LCB) in fg/kg b.w.·d by using the multistage model.

[b]Linear model: $P(d) = 1 - e(-a - bd)$. Cubic model: $P(d) = 1 - e(-a - cd^3)$.

[c]G-O-F, goodness of fit.

[d]Best fit.

[e]A multistage model is fitted to the average liver concentration of TCDD, and safe doses are transformed by using the relationship

Administered dose = liver dose/510.297

The divisor is the least-squares slope between the administered and the average liver dose of TCDD for control and the two lowest doses.

the goodness-of-fit test. When the best fit to the data was nonlinear, the linear model was also fitted (see the note at the bottom of Table 8 for a description of this model) and produced risk estimates for comparison. In all sites, except female liver tumors, the data could be adequately fitted. For males, the smallest lower confidence bound on the "virtually safe dose" (VSD) for an added risk of 1/1,000,000 is 117 fg/kg b.w.·d. In females, the most sensitive site seemed to be the liver, but it was not possible to get an adequate fit using the administered dose. In the original manuscript, Kociba et al. (1978) had determined the concentration of TCDD in the livers of a sample of the sacrificed animals from each dose group. The means for each dose group were:

Administered dose:	0.001	0.01	0.1
Liver concentration (ppb):	0.540	5.10	24.0

Assuming these concentrations were present in the animals at stable levels for much of the study, these are the appropriate doses to which the liver tumor incidence data should be fitted. Assuming the relationship between administered dose and liver dose is nonlinear above the 0.01-μg/kg b.w.·d dose and linear below this dose (as appears to be the case), liver dose can be transformed back to administered dose by using the least-squares line through the points (0,0), (0.001,0.54), and (0.01,5.1). This leads to the linear relationship:

$$\text{Administered dose} = \text{liver dose}/510.297$$

However, the data on liver concentrations may not be sufficiently precise to assume dose-dependent linear or nonlinear relationships. The VSD estimates and lower confidence bounds in the administered-dose scale appear in Table 8 under model type "Transf." With this approach, the smallest lower confidence bound on the VSD by using an added risk of 1/1,000,000 cancers is 27.6 fg/kg b.w.·d for female rats.

The NCI/NTP study (NTP, 1982b) was a gavage experiment on B6C3F1 mice and Osborne-Mendel rats, of both sexes. Seventy-five vehicle-treated control animals and 50 animals were treated at each of 3 doses for each sex by species combination. The doses were administered twice weekly. These weekly doses were divided by 7; thus daily doses were obtained and the same scale could be used. There are, of course, questions of peaks and dips in body content of TCDD. We have assumed that in a weekly scale the dose is approximately constant and that division by 7 to yield daily doses is an acceptable conversion. Both sexes in rats and male mice received doses of 0.0014, 0.0071, and 0.0714 μg/kg b.w.·d (0.01, 0.05, and 0.50 on the weekly dose scale). Female mice received doses of 0.0057, 0.0286, and 0.2859 μg/kg b.w.·d (0.04, 0.2, and 2.0 on the weekly scale). There were no significant survival differences in any group and, in fact, the estimates of the VSD based on a time-to-tumor model (multistage Weibull) were similar to the estimates obtained from the linear model (Crump et al., 1977).

The important lesion sites, the estimates of risk, and the chi-squared goodness-of-fit statistic are given in Table 9. As before, when the linear model was not the best fit, it was fitted separately in order to see what difference this model would make. All of the models gave acceptable fits to the data. Where there were two or more lesion sites for a particular animal group, the EPA pooled the results as mentioned before. Again, independent sites were not combined.

The smallest lower confidence bounds on the VSD (1/1,000,000) for

TABLE 9. Estimates and Approximate 95% Lower Confidence Limits for the VSD[a] of TCDD from the NCI/NTP Study

Lesion	Model[b]	Added risk		Chi-squared G-O-F[c]
		1/10,000	1/1,000,000	
Male rat thyroid				
Follicular-cell adenoma	Linear[d]	40291 (21435)	403 (214)	4.81
Female rat thyroid				
Follicular-cell adenoma	Cubic[d]	7142868 (36711)	1542868 (367)	0.48
	Linear	75737 (34895)	757 (349)	0.79
Female rat liver				
Neoplastic nodules	Linear[d]	1442863 (19298)	105571 (193)	1.31
	Linear	31520 (18784)	315 (188)	1.61
Nodules and carcinomas	Quadratic[d]	1338200 (16523)	133820 (165)	1.31
	Linear	25552 (16001)	256 (160)	1.74
Male mouse liver				
Hepatocellular carcinomas	Linear[d]	26293 (15147)	263 (151)	1.01
Adenomas and carcinomas	Linear[d]	13394 (8633)	134 (86)	0.14
Female mouse liver				
Hepatocellular carcinomas	Linear[d]	246021 (121594)	2460 (1216)	0.74
Adenomas and carcinomas	Linear[d]	14503 (77876)	1450 (779)	2.59
Female mouse thyroid				
Follicular-cell adenoma	Linear[d]	301051 (139090)	3011 (1391)	3.75
Female mouse sarcoma				
Subcutaneous tissue	Linear–cubic[d]	430263 (142803)	4303 (1428)	0.05
	Linear	295289 (142777)	2953 (1427)	0.06
Female mouse lymphoma and leukemia	Linear[d]	102852 (54271)	1028 (543)	0.02

[a] Entries are virtually safe dose (VSD) lower confidence bounds (LCB) in fg/kg b.w.·d by using the multistage model.

[b] Linear: $P(d) = 1 - 3(-a - bd)$. Quadratic: $P(d) = 1 - 3(-a - cd^2)$. Cubic: $P(d) = 1 - 3(-a - fd^3)$. Linear–quadratic: $P(d) = 1 - 3(-a - bd - cd^2)$. Linear–cubic: $P(d) = 1 - 3(-a - bd - fd^3)$.

[c] G-O-F, goodness of fit.

[d] Best fit.

each sex/species combination are as follows: male rat thyroid, 214; female rat liver nodules and carcinomas, 160; male mouse liver adenomas and carcinomas, 86; and female mouse lymphoma/leukemia, 543. These results do not differ markedly from the results of the Kociba study.

Thus, the risk-assessment calculations for the different tumors in these two studies provide a dose range of 280 fg/kg b.w.·d to 14 pg/kg b.w.·d that would result in an increased cancer risk of 1/100,000 and a dose range of 28 fg/kg b.w.·d to 1428 fg/kg b.w.·d that would result in an increased cancer risk of 1/1,000,000. Direct conversions from rodents to

humans were used. It is not clear whether this is justified. Humans repair DNA faster than rodents, and many other differences could be pointed out. None of the extrapolation approaches has at present absolute indisputable scientific justification, including the approach used in this document. However, there is also no scientifically justified alternative form of extrapolation (e.g., use of safety factors) that should preferentially be used. Furthermore, body weight instead of body surface was used, since sizes of organs and, thus, doses to organs correlate better with body weight. Many other biological factors also correlate better with body weight (Krasovisky, 1976).

These calculations assume that a linear dose-response relationship exists for carcinogens that are believed to be primarily promoters. The dose response curve for promoters, however, may not be linear, causing an overestimate of the risk. The model was used on a hypothetical basis and the cancer risk for TCDD should be reevaluated as the data base enlarges. The linear dose-response relationship, however, has not been shown experimentally, and it is not understood how promoters affect cancer growth at very low concentrations. TCDD apparently affects cell membranes through lipid peroxidation (Stohs et al., 1983), which also affects membrane fluidity. The effect of TCDD on membranes is the most likely reason for the formation of multinucleated cells in TCDD-exposed animals (Jones and Butler, 1974; Kimbrough et al., 1977). Most probably, at very low dosage levels, antioxidants such as vitamin E, vitamin C, selenium, and unsaturated fatty acids would have a protective effect against the promoting actions of TCDD (Ames, 1983). Certainly many other naturally occurring and man-made materials also cause lipid peroxidation (Ames, 1983). At doses of TCDD in the picogram range the contribution that this chemical would make might be overriden by the effect of this potpourri of substances and modified by dietary intake of natural antioxidants and thus may not greatly contribute to the risk of cancer or other chronic diseases.

Sweeny et al. (1979) have shown that iron deficiency results in decreased liver toxicity of TCDD. It has also been shown with related chemicals, such as the polybrominated biphenyls, that serosal transfer of iron from duodenal gut sacs of rats is increased. This also occurs *in vivo* in rats with a concomitant rise in serum iron (Manis and Kim, 1980). It is possible that TCDD would have similar effects on iron absorption. For these reasons and, as illustrated by the receptor model developed by Poland, it is not known whether the linear-derived multistage model for assessing cancer risk is the most appropriate. Unfortunately, a scientific data base that would permit the use of different, possibly less conservative models does not exist. Although a different model could be developed based on the receptor theory, it is not entirely clear that all of the toxicity of TCDD is controlled by the *Ah* locus (Sweeny and Jones, 1983).

Since the no-observable-effect levels for reproduction, immune tox-
icity, and various other toxic effects are not established in various species,
a conservative approach for chronic toxicity in general is in order. The
study by Murray et al. (1979) suggested that 0.001 $\mu g/kg \cdot d$ is a
no-observed-effect level for reproduction in rats. Nisbet and Paxton (1982)
recalculated the data developed by Murray et al. (1979), using results from
different generations as independent variables. They concluded that 0.001
$\mu g/kg$ b.w.$\cdot$d was still an effect level. However, the study by Murray et al.
(1979) shows a very varied fertility index among the controls through
different generations; in addition, TCDD body burdens of the dams are
greatly affected by lactation, introducing another variable. CDC con-
sultants Drs. Hoel, Van Ryzin, and Portier also reviewed these data and
concluded that there was insufficient evidence for an effect at 0.001 $\mu g/kg$
b.w.$\cdot$d. For these reasons, this study was not used for risk assessment
calculations, but only the chronic toxicity studies which demonstrated a
carcinogenic response in rodents were used.

Subhuman primates (which are much more susceptible to the effects
of TCDD) show an effect on reproduction if fed for 6 mo at a daily dose
of 1.8 ng/kg. If the toxicology data from subhuman primates are used,
then a 1000-fold safety factor would have to be used, since the lowest
dose of 1.8 ng/kg$\cdot$d was not a no-observed-effect level and was not
obtained from a chronic feeding study. Thus, a daily dose rate of 0.0018
ng/kg—corresponding to a total daily dose of 144 pg—would be tolerable
for an 80-kg person. For a 60-kg person, the tolerable total daily dose
would be 108 pg. Thus, at the daily dose likely to be obtained as
estimated above for a soil level of 1 ppb (44.6 pg/d), these extrapolations
from reproductive studies in subhuman primates appear to suggest a
situation of no excess risk in humans. However, at virtually all other
estimated levels of daily dose (i.e., under more severe sets of assumptions
or the higher level of TCDD in soil), one might expect adverse repro-
ductive health effects.

As shown in Fig. 2, the upper-bound cancer risk estimate of 1.4 pg/kg
b.w.$\cdot$d for an increased risk of 1×10^{-6} over a 70-yr lifetime (assuming
100% of contaminated area is at peak recorded level) is more than the
amount of TCDD (637 fg/kg b.w.$\cdot$d) that could theoretically be absorbed
from soil initially contaminated with TCDD at 1 ppb, under the simula-
tion model as discussed above. For the estimates illustrated in Fig. 2, the
excess lifetime cancer risk for exposure to residential soil with a peak
TCDD contamination level of 1 ppb ranges over 4 orders of magnitude,
from above 10^{-5} to below 10^{-8}. At the lower bound for the VSD of 28
fg/kg b.w.$\cdot$d (or 1.960 pg/70-kg person$\cdot$d), it would take just over 3 yr to
accumulate a total dose sufficient to increase an individual's lifetime risk
of developing cancer by 1 in a million, using the estimates derived herein
and assuming 100% of the contaminated area is at the peak recorded
level). Over a 70-yr lifetime, this would amount to a 0.000023 absolute

001302

increase (equivalent to 0.01% relative increase) over one's "normal" 25% probability of developing cancer in the United States (RR = 25.0023/ 25 = 1.0001).

It must be stressed that the exposure assessments used in estimating risks for carcinogenicity and reproductive health effects contain critical assumptions that are not likely to be actually encountered. Most prominent of these is the assumption of uniform levels of contamination throughout the living space. In fact, areas with elevated TCDD levels are likely to be found in specific, well-defined locations that have concomitant unique use and access characteristics. Therefore, in an area where access is less than total, the actual daily exposure will be lower. Similarly, different usage patterns of affected areas (e.g., sports activities, gardening, horseback riding) or an individual's characteristics (e.g., pica in children) are not likely to lead consistently to worst-case situations and will have differing effects on the determination of total cumulative dose. It could be further argued that the daily dose rate is more important than the cumulative total dose. It appears, however, that exposure has to be for a sufficiently long time for cancer to develop since short exposure periods may result in recovery (Farber, 1974), or would not appreciably increase the cancer risk over background (Office on Smoking and Health, 1982). We have therefore concluded that a soil level of 1 ppb TCDD in residential areas is a reasonable level at which to express concern about health risks.

Implications for Risk Management

Therefore, where residential soil levels exceed 1 ppb, risk-management decisions on habitability and limiting exposure may range from recommendations to avoid identified "hot spots," limit specific activities in these areas (if possible), and to temporarily relocate while contamination clean-up and onsite stabilization operations are performed to permanent relocation and access restriction for a given site. In addition, such recommendations will have to be prepared in terms of situations that range from the need for near-term action to those of a less urgent nature. In all of these scenarios, however, these decisions must be made on a site-specific basis, as indicated by the complexities and variability of circumstances discussed in this document.

Although from these calculations levels of TCDD below 1 ppb are, for practical purposes, considered not to reach a level of concern, several additional considerations related to the risk assessment calculations should be pointed out to decision-makers involved in risk management:

1. The calculations for this level and the judgment that this level is a reasonable level for concern assume that exposure probably will not be consistent for a lifetime, since TCDD will slowly degrade, and that people will not be exposed extensively to the soil on a continuous daily basis. For instance, it is anticipated that during cold or rainy

001303

weather, not much outside activity will occur. From the limited available information, it also appears that levels of TCDD within houses are at least 100-fold less than levels measured outside the houses.
2. The precise bioavailability of TCDD from soil is not known. Such bioavailability may vary with the soil type. It has recently been established that TCDD-contaminated soil from Missouri is toxic to guinea pigs and rats, if given orally. It was estimated that the bioavailability was 30–50% or more (E. E. McConnell et al., 1984).
3. The recovery of TCDD that is extracted from soil for chemical analysis varies a great deal and may be as low as 20%.
4. Fries et al. (1982) have shown that cattle, sheep, and swine consume up to 7% soil/d in their total ingested dry matter when grazing on ranges. Judging from experience with polybrominated biphenyls and 1,2,3,6,7,8-hexachlorodibenzodioxin, levels in the adipose tissue of these animals will probably bioaccumulate (see risk assessment scenario for ranges). Soil levels of TCDD on ranges and other farmland should, therefore, reflect the levels given in Table 10.
5. Furthermore, if contaminated soil is close to waterways and can contaminate these waterways by way of erosion, acceptable levels may also have to be lower, since fish can bioconcentrate TCDD 20,000-fold [National Research Council of Canada (NRCC), 1981] or more. The Food and Drug Administration (FDA) has set concern levels for fish; at 50 ng TCDD/kg edible portion, fish should not be consumed, and at 25–50 ng/kg, fish should not be consumed daily. (Note: The FDA concern levels were established prior to work on this document and reflect both theoretical and practical considerations.)

SCENARIOS

Environmental situations may vary widely, and whether a certain level of TCDD in soil will give rise to concern has to be evaluated on a case-by-case basis. To provide guidance for those who have to deal with these situations, we describe several scenarios of how a level of concern is developed that is appropriate to the specific situation.

The calculations made in Tables 5 and 6 are for residential areas and include children.

Exposure in factories would usually be for a 40-h work week. This situation will be addressed separately by the National Institute for Occupational Safety and Health.

Other areas considered are commercial areas, farmland, and areas that are essentially remote or inaccessible.

Conditions in individual situations may vary considerably and in the end require judgment and common sense.

TABLE 10. Concentrations of TCDD in Soil That Are Projected to
Produce the Maximum Allowable Residues in Foods

Food	TCDD in fat (pg/g)	Observed ratio[a]	Soil (pg/g)
Beef[b]	7.9	0.39	20
Beef (cull dairy)[c]	7.0	0.10	79
Pork	22.7	1.86	12
Milk	2.5	0.40	6.2

[a]Concentration of polybrominated biphenyls (PBB) in product/
concentration of PBB in soil (Fries and Jacobs, 1983).

[b]Includes dairy cattle that have never lactated.

[c]Older cows. Younger cows would approach the values for beef
cattle.

Commercial Areas

At most commercial sites, parking lots and sidewalks are usually either
paved or graveled, and exposure of the general population is transient and
does not occur every day. Usually, children do not play in such areas for
any length of time. In such situations, ingestion would be negligible, and
inhalation and dermal exposure would be the primary routes. When
concrete or gravel is involved, inhalation would also be negligible; thus,
dermal absorption would be the primary exposure route. Since these total
doses for all routes are so much smaller than in residential areas, a level of
concern may not necessarily be reached unless levels are severalfold or
more above 1 ppb. However, if concentrations are high or if there is a
possibility for movement of soil or a dust problem, remedial measures
(such as paving of the area) should be considered in any event. If levels
are very high—e.g., above 100-ppb—more extensive remedial action may
have to be considered.

Farmland

As already stated, the uptake of TCDD from soil by most plants is
negligible. However, further studies are needed to determine whether this
is true for carrots and other root plants (Isensee and Jones, 1971; Coccuci
et al., 1979; Wipf et al., 1982). (This statement does not refer to instances
of direct application of TCDD to plants or crops.) However, erosion into
streams may occur. This might be prevented by leaving several feet at the edge
of the land unplowed and planting grass, shrubs, and/or trees on this edge.

Ranges

The situation is different for ranges where cattle graze, as outlined
next. (Note: This section is based on information and guidance from the

U.S. Department of Agriculture, which is primarily involved in the evaluation of exposures in this setting.)

In this brief assessment, guidance is provided on concentrations of TCDD in soil that would be of concern when animals exposed to that soil are used to produce foods for human consumption. Farm families that use milk from their own cows or slaughter their own animals for home consumption would *probably* receive the highest exposures to TCDD from food (excluding fish). If the products moved off the farm, exposures to the general population would be much lower because they would be diluted with uncontaminated products. This assessment is made for a maximum allowable intake of 100 pg TCDD/d. Proportional adjustments can be made in the assessment for changes in allowable intake or other assumptions, because there is no evidence that the transfer processes for TCDD are affected by concentration.

The assumptions used for average daily beef, pork, and milk consumption are given in Table 11. The values for beef and pork are derived from values for U.S. per capita retail sales of these meats in the past 5 yr. Per capital consumption of veal and lamb is too low to merit serious consideration here. The value used for milk is higher than per capita consumption, but it is a value that has been used frequently in risk assessment, and it is reasonable for some segments of the population, such as teenage boys. The fat contents, daily fat intake, and allowable concentrations of TCDD to maintain intake under 100 pg/d for each of these foods are also given in Table 11.

Data directly relating soil concentrations of TCDD to concentrations of TCDD in milk or tissues of exposed animals are lacking, and these values must be estimated by indirect means. Firestone et al. (1979) fed penta-chlorophenol (PCP) that contained several dioxins to lactating cows for 70 d. The PCP did not contain TCDD, but milk and tissue residue values were obtained for several other dioxins. The most efficiently absorbed dioxin was 1,2,3,6,7,8-hexachlorodibenzodioxin, and its concentration in milk fat was 2.4 times the concentration in the diet at the end of the study. This ratio is much lower than the ratios for many chlorinated hydrocarbons (Fries, 1982), and it is somewhat lower than the 3.1-to-1 ratio for

TABLE 11. Estimated Daily Consumption, Fat Content, and Allowable Concentration of TCDD in Fat to Maintain Human TCDD Intake Below 100 pg/d·person

Food	Consumption (g/d)	Fat (%)	Fat consumption (g/d)	Allowable TCDD in fat (pg/g)[a]
Beef	105	8–12	8.4–12.6	7.9
Pork	54	6–8	3.2–4.3	22.7
Milk	1000	4	40	2.5

[a]Based on the higher fat intake value.

polybrominated biphenyls (PBB) under similar experimental conditions (Fries and Marrow, 1975). Therefore, the results of a study of animal residues on Michigan farms with residual PBB in soils (Fries and Jacobs, 1983) can be used as a reasonable model for assessing movement of TCDD from soil to animals.

The ratios of concentrations of PBB in milk and tissue fat to concentrations in soil are given in Table 10. When the ratios are divided into the maximum allowable TCDD in fat, soil concentrations are obtained that might be expected to produce the maximum allowable concentration in food products. The most serious problem in this assessment is with swine, which consume more soil than other species. Among ruminants, the most serious concern would be from animals that have never lactated.

The values in Table 10 were derived for situations in which animals were confined to limited holding areas. Direct measurements of the type used here are not available for animals on pasture. However, a review (Fries, 1982) of soil ingestion by animals on pasture suggests that the situation would not differ markedly under most conditions, but that milk would become relatively more important as a source of residues.

The values derived in this assessment should not be considered absolute determinants of the suitability of soils for livestock production. Rather, the values should be considered a guide to situations that require a more detailed evaluation.

CHEMICAL ANALYSIS

The available methods for determining 2,3,7,8-TCDD in μg/kg (ppb) or lower concentrations are expensive, time-consuming, and difficult. The nature and use of these analyses necessitate elaborate quality control and quality assurance mechanisms (Crummett and Stehl, 1973).

In the methods used, an isotopically labeled internal standard is added to the sample before the extraction step. Studies have shown that at 12 ppb or greater the final result is independent of the percent recovery of the internal standard. Other studies have shown that the optimum recovery of 2,3,7,8-TCDD from soil is reached only after several extractions. The internal standard may be recovered more efficiently because it is added in a solvent, and, consequently, it may not be bound as tightly to the soil. This latter combination would result in variable and oftentimes erroneously low results for the native 2,3,7,8-TCDD.

Another danger in allowing recovery of an internal standard (which is added at 2.5 ppb) to be low (e.g., 35%, thereby recovering only the equivalent of 0.875 ppb of labeled 2,3,7,8-TCDD) is that the signal/noise ratio for a 1-ppb native 2,3,7,8-TCDD sample would be decreased. If it is decreased to the extent that the signal representing 1 ppb 2,3,7,8-TCDD (for 35% recovery from a 10-g sample, this would equal 3.5 ng in the final extract) is no longer within the linear range, then the percent recovery of

the internal standard and the final result may not be independent. This is especially true for samples with a high background. Because of these reasons and the imprecision associated with other steps in the procedure, analytical results reflect a range rather than a specific number.

RECOMMENDATIONS FOR FURTHER STUDY

Human

- Conduct additional case-control studies to determine the association between exposure to TCDD and related compounds and the incidence of soft tissue sarcomas.
- Conduct epidemiologic studies to characterize and follow populations exposed to TCDD. Determine their TCDD body burdens and the short- and long-term health effects.
- Include immunologic evaluation and evaluation of other likely target organs in epidemiologic studies of TCDD-exposed populations and develop baseline data for these end points.
- Develop better methods to analyze blood and tissue for TCDD at low concentrations. These should include radioimmunoassay (RIA) procedures and aryl hydrocarbon hydroxylase (AHH) induction with biopsy material.
- Develop methods to increase excretion of TCDD after it accumulates in the body.
- Obtain better data on the toxicokinetics of TCDD in humans.
- Analyze human breast milk samples for TCDD and related compounds (to serve as an index for exposure and absorption) in exposed populations.
- Determine soil exposure/day by humans in various settings.

Experimental

- Conduct a series of studies related to the question of bioavailability of TCDD, to focus on differences by the type of soil, by routes of absorption, by species, by aging of soil/dioxin mixture, and by maturity of exposed animals.
- Study TCDD carcinogenicity and reproductive effects in the most sensitive animal species (guinea pig and/or subhuman primates). Carcinogenicity studies should also be conducted in nonsensitive and intermediate species to determine whether the relation between the acute toxicity dose and the carcinogenicity dose is similar for all species.
- Conduct immunologic dose-response studies in young and adult animals.
- Conduct lifetime follow-up studies of immature exposed animals.
- Determine whether the ligand-receptor is integral to the mechanism(s)

of action and search for antagonists and endogenous ligands in different tissues (including human cell systems in tissue culture).

- Determine whether TCDD exerts its carcinogenic potential by an initiator or promoter mechanisms.
- Determine mechanisms for differences in species sensitivity.
- Study the mechanism of TCDD toxicity, particularly for cancer, as it relates to the ligand-receptor theory.
- Determine tissue TCDD levels in animals (guinea pigs) exposed *in situ* to TCDD-contaminated areas, thus providing further information on exposure and bioavailability.
- Prepare polychlorinated dibenzodioxin isomer standards for future laboratory work.
- Establish the role of metabolism more clearly.
- Establish the interactions of ligand-receptor complexes with macromolecules in hopes of ascertaining functional differences in these interactions between "sensitive" and "nonsensitive" species. These findings could be used in human risk assessment by providing a direct relationship between exposure and mechanism of toxicity.

Environmental

- Develop utilizable methods for detoxifying TCDD-contaminated soil.
- Determine ambient (background) levels of TCDD in soil, air, water, etc. (as well as in people).
- Establish "sentinel" animals as indicators of the bioavailability of TCDD in contaminated areas.

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