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HEALTH ASSESSMENT DOCUMENT FOR DIOXINS

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Prepared by

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NOTICE

This document is a preliminary draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comments on its technical accuracy and policy implications.

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2. SUMMARY AND CONCLUSIONS

2.1. SUMMARY

Most of the relevant physical properties of the four polychlorinated dioxins discussed in this document presently remain scientifically undetermined. The solubility of 2,3,7,8-TCDD in water is 0.2 µg/ℓ. This isomer and the three other polychlorinated dioxins are more soluble in aromatic solvents than in aliphatic solvents. The polychlorinated dioxins are chemically very stable and decompose at temperatures >800°C.

The general method for the determination of these compounds in different samples consists of solvent extraction, followed by sulfuric acid and base washes to remove lipids and other impurities from the solvent extract. The extract is then subjected to two liquid chromatographic clean-up procedures. The cleaned up extract is finally analyzed for the polychlorinated dioxins by the gas chromatographic-mass spectrometric method. Despite the specialized methods used for the determination of polychlorinated dioxins, the results of analysis at very low levels (possibly <9 parts per trillion) still remain questionable.

None of the polychlorinated dioxins are either commercially manufactured or have any known use. They are produced as unwanted contaminants during the manufacture of, primarily, chlorophenols and their derivatives. The primary sources of the polychlorinated dioxins in the environment are industrial manufacture of chlorophenols and their derivatives and the chemical disposal sites containing the wastes from these industries. Although municipal incineration may produce some environmental emission of polychlorinated dioxins, the available data indicate that this source is far less significant than industrial emissions and emission from certain chemical disposal sites.

The monitoring data to date indicate that the maximum level of polychlorinated dioxins is likely to be found in soil and drainage sediment samples near chlorophenol manufacturing industries and chemical waste disposal sites. With the exception of air near certain contaminated sites, none of the polychlorinated dioxins have been detected in U.S. air samples. Small amounts of these compounds have been found in fish and wildlife in the U.S. in areas around chlorophenol manufacturing industries and certain chemical waste disposal sites.

The environmental fates of the four polychlorinated dioxins are not known with certainty. Most of the investigations in this field have been conducted with 2,3,7,8-TCDD, and the conclusions regarding the environmental fate of the other three polychlorinated dioxins have been drawn by analogy. Few data exist in the literature that would indicate significant chemical and biological transformation of these compounds in atmospheric, aquatic, or soil media. The role of photochemical transformation in determining the fates of these chemicals in various media is not known with certainty. In aquatic media, a substantial proportion of the polychlorinated dioxins may be present in the sediment-sorbed state or in the biota. In the atmosphere, the polychlorinated dioxins are expected to be present in the particulate-sorbed state. The atmospheric transport of these compounds can be predicted from dispersion modeling equations. In the case of the accidental release of a toxic cloud containing 2,3,7,8-TCDD at Seveso, Italy, it has been experimentally demonstrated that 2,3,7,8-TCDD deposition from air to soil follows an exponential decay pattern along the downward wind direction. The two most probable transport mechanisms of the polychlorinated dioxins from soils are the transport to atmosphere via contaminated dust particles and to surface water via eroded soil.

Both the calculated and the experimental results show that the polychlorinated dioxins will bioconcentrate in sediments and biota present in

aquatic media. It has been shown by static test procedures that, depending on the species, the bioconcentration factor for 2,3,7,8-TCDD ranges from ≈2000-30,000.

In mammals, 2,3,7,8-TCDD is readily absorbed through the gastrointestinal tract, and asborbtion through intact skin has also been reported. Absorption may decrease dramatically if 2,3,7,8-TCDD is adsorbed to particulate matter such as activated carbon or soil. After absorption, 2,3,7,8-TCDD is distributed to tissues high in lipid content; however, in many species, the liver is a major storage site. Metabolism of 2,3,7,8-TCDD occurs slowly, with the polar metabolites excreted in the urine. Unmetabolized 2,3,7,8-TCDD is eliminated in the feces, although excretion in the milk of lactating rats has also been reported.

The PCDDs discussed in this document are among some of the most toxic compounds known, with the LD_{50} level for guinea pigs being 0.6 µg/kg for 2,3,7,8-TCDD. The other congeners are somewhat less toxic; however, the LD_{50} values are still in the µg/kg range. Although 2,3,7,8-TCDD is highly toxic in all species tested, there are large differences in sensitivity, with the LD_{50} for hamsters being in the low mg/kg range. The characteristic symptoms of lethal poisoning are severe weight loss and thymic atrophy. Death usually occurs many days after the exposure. In rats and mice, 2,3,7,8-TCDD produces severe liver injury which is not observed in either monkeys or guinea pigs, and in mice, the immune response is suppressed. After subchronic or chronic exposure to 2,3,7,8-TCDD in rats or mice, the liver appears to be the most severely affected organ, although hemorrhage, edema, and thymic activity are also observed. The limited data available for the other PCDDs indicate that these chemicals produce the same symptoms as 2,3,7,8-TCDD in a given species; however, the doses required are higher.

Humans have been exposed to herbicides and other chlorinated chemicals containing 2,3,7,8-TCDD as a contaminant. The symptoms of toxicity in many cases are similar to those observed in animals, with exposure leading to altered liver function, porphyria cutenea tarda, and pathologic changes in hematologic parameters. In addition, exposure of humans to 2,3,7,8-TCDD produces skin lesions such as chloracne and hyperpigmentation. Although some symptoms such as chloracne are attributed solely to the PCDDs, the other signs of toxicity may arise, at least in part, from the parent chemical of which PCDDs are a minor contaminant.

Animal studies have demonstrated that 2,3,7,8-TCDD is teratogenic in rats, mice, and rabbits, and fetocidal in monkeys. Exposure to 2,3,7,8-TCDD in mice produces cleft palates, while exposure in rats results in edema, hemorrhage, and kidney anomalies, and rabbits had a higher incidence of extra ribs. Human epidemiology studies neither confirm nor refute the teratogenic potential of 2,3,7,8-TCDD. Some studies have shown positive associations with exposure and birth defects and abortions, while others have not.

Although there is only limited and conflicting evidence that 2,3,7,8-TCDD is a mutagen or produces chromosmal damage, a number of chronic animal bioassays show that the compound is an animal carcinogen. In rats, oral exposure to 2,3,7,8-TCDD results in adenomas or carcinomas of the thyroid, hepatocellular carcinomas, carcinomas of the tongue, carcinomas of the hard palate, and adenomas of the adrenal. In mice, increased incidence of liver tumors was observed. A mixture of the two congeners of HCDD has also been tested for carcinogenicity and shown to produce increased incidences of liver tumors in rats and mice. Also, 2,3,7,8-TCDD has produced papillomas after dermal administration, while there was no significant increase in dermal tumors when the mixture of HCDD was tested. Since both compounds produce increased incidences of tumors in two species of

animals, there is sufficient evidence to indicate that these compounds are animal carcinogens. Although some evidence from human studies associates exposure to herbicides contaminated with 2,3,7,8-TCDD with human cancers, the exposures were always mixed, making it difficult to assert that 2,3,7,8-TCDD was the sole active agent.

2.2. CONCLUSIONS

The PCDDs discussed in this document, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 1,2,3,7,8,9-, 1,2,3,6,7,8-HCDD, are highly toxic following acute exposure. All animal species administered high levels of these compounds developed severe weight loss and thymic atrophy. In some species, liver damage, edema, hair loss, and immunosuppression were also observed. Chronic toxicity studies have been conducted only on 2,3,7,8-TCDD and a mixture of the two congeners of HCDD. In these studies, the primary non-neoplastic lesion was fatty and necrotic change in the liver.

The fetus has been shown to be highly sensitive to the toxic effects of 2,3,7,8-TCDD. In rats, the malformations observed included hemorrhage, edema, and kidney anomalies, while in mice, the predominant lesions were cleft palate and kidney anomalies. The lowest reported exposure, 0.001 µg/kg, produced a significant effect on the fetus, was similar to the NOEL observed in chronic studies.

Evidence from animal bioassays is sufficient to indicate that 2,3,7,8-TCDD and a mixture of the two congeners of HCDD are animal carcinogens. 2,3,7,8-TCDD has increased the incidence of a variety of tumors in rats and hepatocellular tumors in mice, while the mixture of HCDD tested increased the incidence of hepatocellular tumors in both rats and mice. There were no chronic studies to determine the carcinogenic potential of 1,2,3,7,8-PeCDD. Although epidemiology studies have associated exposure to chemicals contaminated with PCDDs with

increased tumor incidence in man, the mixed exposure makes this evidence insufficient to demonstrate that any of the PCDDs are human carcinogens.

2.3. NEEDS FOR FUTURE RESEARCH

- The basic physical properties such as water solubilities and vapor pressures of the pentachloro- and hexachlorodibenzo-p-dioxins need to be determined. These parameters are important in predicting the environmental fate of these compounds.
- New analytical methodologies must be established to determine the low levels of these compounds in environmental matrices without ambiguity.
- More monitoring data, particularly in air and aquatic media, should be developed by a diversity of research groups. The predominant amount of monitoring data in the U.S. originate from one source. These data need to be independently confirmed.
- More research efforts should be directed to determining the environmental fate of the pentachloro- and hexachlorodibenzo-p-dioxins. The determination of the fate of these chemicals with respect to the possibility of photochemical transformations in different environmental matrices needs special attention.
- Pharmacokinetic studies should be conducted to demonstrate more clearly the degree of absorption of the PCDDs by all routes. In particular, studies are needed on respiratory absorption and on PCDD adsorbed to environmental media.
- Although a number of studies demonstrate that 2,3,7,8-TCDD is a teratogen, the other congeners should be tested for teratogenic potential.
- There is no information on the effects of chronic exposure to 1,2,3,7,8-PCDD, and studies should be conducted to determine both the toxic effects of this compound and its carcinogenic potential.

- Further epidemiology data on the effects in human populations exposed to PCDDs would assist in determining which effects observed in animals are also present in humans. In these studies, careful quantitation of PCDD levels would provide dose-response data necessary for health assessment.

11.4 SUMMARY AND CONCLUSIONS

Qualitative Assessment-TCDD

Probably one of the most toxic chemicals known to man is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

In small amounts, TCDD is a potent inducer of arylhydrocarbon hydroxylase in mammals. This is a complex enzyme system that consists of epoxidase, epoxidehydratase, and glutathione transferase. The enzyme epoxidase is known to mediate the formation of epoxides, which are potentially active carcinogenic metabolites. TCDD can be metabolized in mammalian species via the epoxide to dihydrodiol and further conjugates with glutathione. Persistent residues of TCDD were found in liver and fat in a 2-year feeding study in rats. Significant covalent binding of TCDD to protein has been demonstrated by two investigators. Covalent binding of TCDD with DNA is not significant in liver cells.

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

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

The 1978 study by the Dow Chemical Company of male and female Sprague-Dawley rats fed TCDO in doses of 22 ppt, 200 ppt, and 2200 ppt revealed a highly statistically significant excess incidence of hepatocellular carcinomas in female rats at the highest dose level and hepatocellular carcinomas and hepatocellular hyperplastic nodules in female rats at the middle dose level, as compared to the controls. In addition, there was a significant increase in carcinomas of the hard palate/nasal turbinates in both high dose males and females, of the tongue in males, and of the lung in females. The Van Miller et al. (1977) study also showed some evidence of a carcinogenic response in the liver and lungs of male Sprague-Dawley rats at dosages of 1000 and 5000 ppt in the diet, even though the study used a relatively small number of animals. The Toth et al. (1979) study provides suggestive evidence that TCDD induced an increased incidence of liver tumors in male mice (females were not tested) receiving 0.7 ug/kg/week by gavage.

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

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

carcinomas in the high-dose males and females, and thyroid tumors, subcutaneous fibrosarcomas, and histiocytic lymphomas in females.

In the study by Pitot et al. (1980), TCDD has been shown to be a potent liver cancer promoter after initiation with diethylnitrosamine. Several tests of TCDD as a promoter on mouse skin were negative, but Poland et al. (1982) showed that TCDD can promote in one mouse strain and is negative in others. In the study by Kouri et al. (1978), TCDD has been shown to be a potent co-carcinogen with 3-methyl chloranthrene.

Several epidemiological studies have been conducted which are relevant to the assessment of the carcinogenicity of 2,4,5-T, silvex, and TCDD. Two Swedish epidemiological case-control studies (Hardell and Sandstrom, 1979; Eriksson et al., 1979, 1981) reported a significant association between soft-tissue sarcomas and occupational exposure to phenoxyacetic acid herbicides and/or chlorophenols. These studies indicated approximately five- to sevenfold increases in the risk of developing soft-tissue sarcomas among people exposed only to phenoxyacetic acids in comparison to people not exposed to these chemicals. When an attempt was made to separate exposures into two categories based on expected presence or absence of polychlorinated dibenzodioxin and dibenzofuran impurities, the relative risks were 17 and 4.2, respectively. This indicates that agents themselves without the dioxin impurities may be contributing to the risk of soft-tissue sarcomas as well. Another Swedish case-control study (Hardell et al., 1980, 1981) provides suggestive evidence of an increased risk of developing lymphomas resulting from occupational exposure to phenoxyacetic acids.

Two cohort studies, one by Axelson et al. (1980) and the other by Thiess and Frentzel-Beyme (1978) provide suggestive evidence that phenoxyacetic acids

and/or TCDD increase the risk of stomach cancer in humans. Four other cohort studies by Ott et al. (1980), Riihimaki et al. (1978), Cook et al. (1980) and Zack and Suskind (1980), did not indicate a significantly increased risk of cancer in people exposed to phenoxyacetic acids and/or chlorophenols, but two of these studies were of relatively low statistical power, and the fourth study has certain inconsistencies requiring clarification.

Qualitative Assessment-HCDD

Hexachlorodibenzo-p-dioxin has also been tested for carcinogenicity in rats and mice treated by gavage and by dermal application to mice (NTP 1980c,d). In these studies, a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD was tested. In the oral study, animals received HCDD at doses of 0.0, 1.25, 2.5, or 5.0 ug/kg/week, except for female mice, which received 0.0, 2.5, 5.0, and 10.0 ug/kg/week. In both species and both sexes, only tumors of the liver occurred at a significantly greater incidence than in controls. In male rats and male and female mice, the liver tumor incidence was significantly increased over control values only in the high-dose groups, while in female rats the incidence was significantly greater at both the medium and high dose levels. In the study of HCDD carcinogenicity in mouse skin conducted by NTP (1980d), there were no treatment-related tumors in either the carcinogenicity bioassay or the tumor promotion assay using DMBA as an initiator.

Quantitative Assessment

No epidemiological studies are suitable for estimating a TCDD inhalation risk to humans. Several animal data sets are available for estimating an inhalation unit risk for TCDD, but they are all based on either gavage or

feeding studies. The quantitative cancer unit risk estimate is based on the Kociba et al. (1978) TCDD feeding study in female rats (histopathologic evaluation by Dr. Robert Squire) that induced a statistically significant incidence of tumors in the liver, lungs, and hard palate or nasal turbinates. Based on continuous lifetime exposure to 1 pg/m^3 2,3,7,8-tetrachlorodibenzo-p-dioxin in ambient air, the estimated upper-limit probability of individual cancer risk is 9.1×10^{-5}.

An upper-limit unit risk estimate for a mixture of HCDDs has also been calculated from the NCI gavage study (NTP, 1980c). Based on combined liver neoplastic nodules and hepatocellular carcinomas in female rats, a continuous lifetime exposure to 1 pg/m^3 of HCDD is estimated to yield an upper-limit unit risk of 1.2×10^{-5}.

The potency of TCDD using the linearized multistage model is also estimated relative to 53 other chemicals which the CAG has evaluated as suspect carcinogens. This relative potency index is $1 \times 10^{+8}$ (mMol/kg/day)$^{-1}$, making TCDD the most potent animal carcinogen, by far, that the CAG has evaluated. It is 100 times more potent than the next most potent chemical, benzidine, and 100,000,000 times more potent than vinyl chloride. The relative potency for HCDD is $2 \times 10^{+7}$, making it the second most potent carcinogen.

Conclusion

Because of the induction of hepatocellular carcinoma in two strains of female rats and both sexes of one mouse strain, along with the induction of thyroid tumors, subcutaneous fibrosarcomas, and lung and tongue tumors in both rats and mice, the evidence of carcinogenicity for TCDD in animals would be

regarded as sufficient if the classification system of the International Agency for Research on Cancer (IARC) were used. These effects occur at extremely low doses. The demonstration of a promotion effect in rat liver after initiation with diethyl nitrosamine and a cocarcinogenic response when TCDD was injected simultaneously with 3-methyl chloranthrene increases the level of evidence in animals.

The human evidence for the carcinogenicity of TCDD alone is regarded as inadequate using the IARC classification, because of the difficulty of attributing the effects to TCDD, which occurred as an impurity in the phenoxyacetic acids and chlorophenols to which the people were exposed.

Hepatocellular carcinomas have been induced in mice and rats following administration of a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD. This level of carcinogenic evidence in animals would be regarded as "sufficient" according to the IARC classification scheme. Therefore, based on animal evidence, HCDD would be placed in Group 2B, which IARC characterizes as probably carcinogenic in humans.

However, the human evidence for the carcinogenicity of chlorinated phenoxy acetic herbicides and/or chlorophenols with chlorinated dibenzodioxin and dibenzofuran impurities is limited according to the IARC criteria. Therefore the overall evidence of carcinogenicity, considering both animal and human studies, would place TCDD alone in the 2B category of IARC, and TCDD in association with the phenoxy herbicides and/or chlorophenols in the 2A category. The IARC regards chemicals in both categories as probably carcinogenic in humans.

13. REGULATIONS AND STANDARDS

13.1. WATER

13.1.1. Ambient Water. Previous release of dioxin-containing herbicides has allowed these agents to enter the environment. Their high environmental stability and low water solubility (0.2 ppb) make the 2,3,7,8-TCDD tend to settle in the bottom sludge of waterways. The major risk to humans comes from eating bottom-feeding fish in which 2,3,7,8-TCDD has bioaccumulated. The U.S. EPA is considering setting criteria of 2.1×10^{-9}, 2.1×10^{-10} or 2.1×10^{-11} ug 2,3,7,8-TCDD/ℓ based on estimated human lifetime cancer risks of 10^{-5}, 10^{-6} or 10^{-7}, respectively. These criteria are based on the assumption of a daily consumption of 6.5 gm contaminated fish and shellfish with or without the additional daily consumption of 2 ℓ of contaminated drinking water (U.S. EPA, 1981). No information is available regarding concentration limits of 1,2,3,7,8-PCDD, 1,2,3,7,8,9-HCDD or 1,2,3,6,7,8-HCDD in ambient water.

13.1.2. Drinking Water. In the criteria proposed by the U.S. EPA, the daily consumption of drinking water containing these levels of 2,3,7,8-TCDD is believed to be without consequence. Only 0.3% of the total 2,3,7,8-TCDD exposure is assumed to occur from drinking water. No information is available regarding concentration limits of other dioxin congeners in drinking water.

13.2. AIR

Many normal combustion processes are suspected of releasing dioxins to the atmosphere. However, the effect on human health from this source is unknown, and no criteria exist regarding concentration limits.

13.3. FOOD

According to the FDA (1981) and (41 CFR 321) fish with a 2,3,7,8-TCDD content averaging ≤25 ppt pose no serious health concern. Federal legal limits for Great Lakes fish distributed in interstate commerce are deemed unnecessary because most of the samples analyzed by the FDA contained <25 ppt. Canada has established a 20 ppt concentration limit for 2,3,7,8-TCDD in Lake Ontario commercial fish imported into the United States to comply with the levels believed by the FDA to be safe.

A tolerance for hexachlorophene methylenebis (2,3,6-trichlorophenol) in or on feedstock cottenseeds has been established at 0.05 ppm, with the condition that it not contain >0.1 ppm of 2,3,7,8-TCDD (U.S. EPA, 1982c).

No information regarding concentration limits of other dioxin isomers is available.

13.4. SUMMARY

The regulation of dioxin by-products in substances such as hexachlorophene and 2,4,5-trichlorophenoxyacetic acid is apparently expected to eliminate dioxin releases to the environment. The Canadian concentration limit for 2,3,7,8-TCDD in fish is the only known criterion, and it agrees with levels regarded by the FDA as being protective of human health. In the absence of specific guidelines and standards regarding concentration limits of 2,3,7,8-TCDD, the FDA examines individual contamination situations separately, and gives only general guidance regarding relative risk to humans (Delgado, 1983). No information is available regarding concentration limits for other dioxin isomers.

14. EFFECTS OF MAJOR CONCERN AND HEALTH HAZARD ASSESSMENT

Of the four congeners of PCDD discussed in this report (i.e., 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,7,8,9- and 1,2,3,6,7,8-HCDD), the majority of toxicologic data are on 2,3,7,8-TCDD. The limited data on the other congeners indicate that they are qualitatively similar in their toxic action to 2,3,7,8-TCDD when comparisons are made in a single species; however, they are less toxic than the 2,3,7,8-TCDD congener. This is illustrated in mice, in which 2,3,7,8-TCDD has an LD_{50} value of 0.88 μmol/kg and 1,2,3,7,8-PeCDD; 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD have, respectively, LD_{50} values of 0.94, 3.19 and 3.67 μmol/kg (McConnell, 1978a). This suggests that either the position or the number of chlorine effects the toxicity of the PCDDs.

In more recent studies using biochemical endpoints, Poland et al. (1979), Bradlaw and Casterline (1979) and Bradlaw et al. (1980) have supported the contention that the position and number of chlorines on TCDD, PeCDD and HCDD are critical for the biologic activity of the compound. In this study, the ED_{50} for the induction of AHH activity in hepatoma cells in culture was used to establish a range of potency for congeners of PCDD. Although acute toxicity and induction of AHH activity have been used to quantify the difference in the biologic activity of the congeners 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,7,8,9-HCDD, the extrapolation of this data to estimate quantitative dose-response relationships for the chronic toxicity of individual congeners are not sufficiently supported at the present time. From the data described below, it is clear that sufficient information for quantitative hazard assessment is available only for 2,3,7,8-TCDD and a mixture of the two HCDD congeners.

14.1. PRINCIPLE EFFECTS

14.1.1. Toxicity. The principle effect observed in all species after acute exposure to 2,3,7,8-TCDD is weight loss and thymic atrophy (see Table 8-1). The decrease in weight proceeds over a protracted length of time even after a single exposure to a lethal dose. By the time of death, an almost complete absence of body fat stores often was observed. At death, severe deterioration of the animal was observed; however, there was no specific lesion to associate with the cause of death. This was particularly evident in the guinea pig, the most sensitive species to 2,3,7,8-TCDD toxicity. Necropsy revealed no remarkable alteration in any internal organ except for thymic atrophy (Gupta et al., 1973). Although liver damage was observed in rats, rabbits and mice (Schwetz et al., 1973), there are insufficient data to indicate that this effect is the underlying cause of mortality after acute exposure to 2,3,7,8-TCDD. Also, in the guinea pig and monkey, which have the same general progression of gross signs of toxicity as do rats, rabbits, and mice, there is only mild liver damage (Section 8.1). In addition, 2,3,7,8-TCDD is an immune suppressant in mice (Section 8.1.1.4); however, again, it is not clear whether immunosuppression is a cause or an effect of the gross symptoms of 2,3,7,8-TCDD toxicity.

As a result of the long time necessary for the development of toxic symptoms in animals, subchronic and chronic studies are better able to define dose effect and response relationships than are acute studies. Subchronic and chronic animal studies that define NOELs and LOELs are summarized in Table 14-1 for orally administered 2,3,7,8-TCDD. The NOEL for subchronic exposure is ≈10 times higher than that observed for chronic exposures, suggesting that the cumulative dose might be an important factor in 2,3,7,8-TCDD toxicity. There are only limited data on the NOEL and LOEL for HCDD (Table 14-2) and these were obtained from studies using a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD. As observed

TABLE 14-1

No Observed Effect Levels and Low Observed Effect Levels Obtained from Subchronic and Chronic Oral Toxicity Studies of 2,3,7,8-TCDD

Species/Strain	pg/kg/day NOEL	LOEL	Duration of Exposure	Duration of Study	Reported Effect	Reference
rat/Sprague-Dawley	0.01	0.1	13 weeks	26 weeks	decreased body weight	Kociba et al., 1976
rat/Osborne-Mendel	0.07	0.14	13 weeks	13 weeks	toxic hepatitis	NTP, 1980a
rat/Sprague-Dawley	0.0014	0.014	16 weeks	80 weeks	elevated porphyrin levels	Goldstein et al., 1982b
rat/Sprague-Dawley	ND	0.014	28 weeks	80 weeks	fatty changes in the liver, decreased body weight	King and Roesler, 1974
mice/B6C3F1	ND	0.014	13 weeks	13 weeks	toxic hepatitis	NTP, 1980a
monkey/rhesus	ND	2	36 weeks	52 weeks	panocytopenia	Allen et al., 1977
rat/Sprague-Dawley	0.001	0.01	104 weeks	104 weeks	degenerative and necrotic changes in the liver	Kociba et al., 1978a, 1979
rat/Osborne-Mendel	0.0014	0.007	104 weeks	107 weeks	toxic hepatitis	NTP, 1980a
mice/B6C3F1	ND	0.001	104 weeks	107 weeks	dermatitis and amyloidosis	NTP, 1980a

ND = Not determined

TABLE 18-2

No Observed Effect Levels and Low Observed Effect Levels Obtained from Subchronic and Chronic Oral Toxicity Studies of HCDD

Species/Strain	mg/kg/day NOEL	LOEL	Duration of Exposure	Duration of Study	Reported Effects	References
rat/Osborne-Mendel	0.35[a]	0.7	13 weeks	13 weeks	hepatotoxicity	NTP, 1980b
mice/B6C3F1	0.7[a]	1.4	13 weeks	13 weeks	hepatotoxicity	NTP, 1980b
rat/Osborne-Mendel	ND	0.18	104 weeks	107 weeks	toxic hepatitis	NTP, 1980b
mice/B6C3F1	ND	0.18	104 weeks	107 weeks	toxic hepatitis	NTP, 1980b

[a]It was not clear whether this dose represented a NOEL or a NOAEL.

ND = Not determined

with 2,3,7,8-TCDD, there is a suggestion that the cumulative dose of this mixture is an important consideration in defining a NOEL. For both 2,3,7,8-TCDD and the mixture of HCDD, the liver appeared to be the most sensitive target organ.

2,3,7,8-TCDD has been shown to produce fetal anomalies in rats, mice, and rabbits (see Table 9-2). In mice fetuses, 2,3,7,8-TCDD induces cleft palate and kidney malformations, while in rat fetuses, hemorrhage, edema and a number of anomalies were observed. There was only one study available assessing the teratogenicity of 2,3,7,8-TCDD in rabbits reported by Giavini et al. (1982b) in which increases in extra ribs and total soft tissue anomalies were observed. In mice, 1 μg/kg given for 9-10 days during the middle of gestation was the minimum dose necessary to elicit a teratogenic response (Smith et al., 1976; Moore et al., 1973), while dilated renal pelvis and decreased fetal weight were observed in the rat fetuses of dams receiving doses of 2,3,7,8-TCDD as low as 0.001 μg/kg throughout gestation. The statistical significance of effects at this later dose, however, is argued (Murray et al., 1979; Nisbet and Paxton, 1982). The fetuses of rats appear to be very sensitive to the effects of 2,3,7,8-TCDD, with adverse effects occurring at maternal exposures that were similar to the NOEL observed in chronic studies (see Table 14-1). Also, Schwetz et al. (1973) demonstrated that HCDD (isomers not specified) was both fetotoxic and teratogenic when administered to pregnant rats at 100 μg/kg on days 6-15 of gestation.

Some epidemiology studies have shown a positive association between exposure to 2,4,5-T, of which 2,3,7,8-TCDD is a known contaminant, and birth defects or abortions. Other studies have failed to demonstrate an association (Section 9.2), while no substantial association with reproductive difficulties has been reported in human populations exposed to 2,3,7,8-TCDD as a contaminant of TCP. These studies in humans can neither support nor refute the animal teratogenicity

data, since exposures were always mixed, and there were inadequate data concerning the levels of 2,3,7,8-TCDD to which the populations were exposed.

Animal studies also demonstrate that 2,3,7,8-TCDD is a carcinogen (see Table 11-1). The limited studies by Van Miller et al. (1977a,b,) and Toth et al. (1978, 1979) indicated that 2,3,7,8-TCDD caused a variety of tumors in rats and mice, and the more intensive studies by Kociba et al. (1978) and NTP (1980a) support these early findings. Also, papillomas have been reported in female mice after dermal application of 2,3,7,8-TCDD (NTP, 1980b), and using the skin tumorigenesis model, it has been shown that 2,3,7,8-TCDD may affect the carcinogenic potential of other chemical carcinogens (see Section 11.1.3). Human exposure to 2,3,7,8-TCDD has resulted from contamination of other polychlorinated compounds with 2,3,7,8-TCDD (see Section 11.2). Although populations exposed to 2,3,7,8-TCDD have been shown to have a higher risk of developing cancer, these studies are only suggestive because 2,3,7,8-TCDD exposure always occurs simultaneously with exposure to other compounds. Also, the human epidemiology studies do not provide sufficient exposure data to define a dose-response relationship.

A 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD also has been tested for carcinogenicity in rats and mice treated by gavage and by dermal application in mice (NTP, 1980c,d). In both species, this mixture produced liver tumors when administered by gavage, while in the dermal study there was no increase in the incidence of skin tumors.

14.1.2. Mutagenicity. There have been many studies of the mutagenic potential of 2,3,7,8-TCDD (see Section 10). <u>In vitro</u> assays using bacteria and yeast have generally indicated that 2,3,7,8-TCDD is not a mutagen. These negative results were obtained both in the presence and absence of a mammalian metabolic activation system. A few studies have reported positive results (Hussain et al., 1972;

Seiler, 1973; Bronzetti et al., 1980); however, these positive studies had deficiencies in either experimental design, or were reported only qualitatively with inadequate description of experimental detail for evaluation. With the available data, it is impossible to assert whether or not 2,3,7,8-TCDD is devoid of mutagenic potential. There are also some conflicting data from humans and animal studies that indicate that 2,3,7,8-TCDD causes chromosomal aberrations. Because the human data are derived from populations in which exposure to other biologically active compounds is possible, and because the increases observed in animal studies were small, it is still not substantiated that 2,3,7,8-TCDD produces clastogenic changes.

There is no information available on the mutagenic potential of 1,2,3,7,8-PeCDD or 1,2,3,7,8,9-, 1,2,3,6,7,8-HCDD.

14.2. SENSITIVE POPULATIONS

Although there are no data from human studies to indicate the presence of sensitive populations, the data from animal studies suggest that the fetus and newborn may be at greater risk. Studies in rats, mice, rabbits and monkeys have shown that *in utero* exposure to 2,3,7,8-TCDD can result in malformations, fetal toxicity and abortions (see Table 9-2). The lowest dose reported to adversely affect the fetus *in utero* was 0.001 µg/kg/day administered to the dams throughout gestation (from Murray et al., 1979, according to Nisbet and Paxton, 1980); this dose is similar to the NOEL reported for chronic exposure of adult rats (see Table 14-1). Moore et al. (1973) have observed that the nursing of pups on mothers exposed to 2,3,7,8-TCDD could also result in kidney anomalies detected at the time of weaning. These data suggest that both the fetus and the newborn may be more sensitive than the adult to the adverse effects of exposure to 2,3,7,8-TCDD.

In addition, 2,3,7,8-TCDD is known to be a powerful inducer of the MFO system. There is information to indicate that MFO induction by 2,3,7,8-TCDD can affect the biologic activity of other xenobiotics that require metabolic activation (see Section 12). Scarpelli et al. (1980), for example, demonstrated that pretreatment of hamsters with 2,3,7,8-TCDD resulted in greater activation of mutagenic nitrosamines when assayed in vitro with isolated microsomes. Individuals exposed to chemicals that are activated by the MFO may experience a synergistic effect and be at greater risk.

14.3. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT

It is expected that the PCDDs discussed here would be highly persistent compounds in the environment, and that human exposure may occur through ingestion of contaminated food and water, by inhalation of the compound absorbed to respirable particulates, or through dermal contact. Although potential exposure may occur by all routes, most of the toxicologic information is from studies of oral exposure. The limited observation of toxic effects in humans and animals after dermal contact with 2,3,7,8-TCDD in organic solvents indicates that dermal absorption occurs. Poiger and Schlatter (1980) have shown in rats that both dermal and gastrointestinal absorption is dependent on the vehicle. Greatest absorption after oral exposure occurred when 2,3,7,8-TCDD was administered in organic solvent followed by aqueous suspension, with little absorption occurring if the 2,3,7,8-TCDD was adsorbed onto activated carbon. In a similar manner, dermal absorption was poor if the 2,3,7,8-TCDD was applied in a soil and water paste. Since inhalation exposure is likely to occur through airborn particulate matter containing absorbed 2,3,7,8-TCDD, it is not possible with the available data to predict how efficiently absorption will occur through the respiratory tract. The use of standard respiratory absorption assumptions in risk assessment are most likely to provide conservative criteria levels.

14.4. QUALITATIVE HEALTH HAZARD ASSESSMENT

The data available from human and animals studies are sufficient to provide some assessment of the health hazards associated with exposure to 2,3,7,8-TCDD and a mixture of 1,2,3,7,8,9- and 1,2,3,6,7,8-HCDD. The only data available on 1,2,3,7,8-PeCDD are an acute LD_{50} value and studies of induction of AHH activity. Although both types of data indicate that 1,2,3,7,8-PeCDD might have slightly less biological activity than 2,3,7,8-TCDD, the data are insufficient to adequately predict the risk associated with a particular dose of 1,2,3,7,8-PeCDD. This would be the case if attempts were made to use this data from acute exposure to extrapolate to effects of chronic exposure whether these effects are toxic or carcinogenic. For the other PCDDs discussed, the hazard assessment can be based on toxicity, teratogenicity or carcinogenicity.

Although there have been human epidemiology studies investigating the toxic, reproductive, and carcinogenic effect of exposure to 2,3,7,8-TCDD, these studies have major deficiencies for use in health assessment. 2,3,7,8-TCDD is a contaminant of the chemicals 2,4,5-T and TCP, and all human data are derived from populations exposed to mixtures. In these studies, it is not possible to attribute with certainty any observed effect to exposure to 2,3,7,8-TCDD. Also, exposure data of sufficient quality data are not available to define dose response relationship in human population. Without adequate exposure data, health assessments cannot be made.

14.4.1. Animal Toxicity Data. Animal studies that are useful for hazard assessment are studies with adequate experimental design to define the levels of exposure that produce threshold effects. Tables 14-1 and 14-2 summarize these studies, providing data on NOEL (or NOAEL) and LOEL (or LOAEL). Since there is suggestive evidence that the cumulative dose is important to the toxicity of 2,3,7,8-TCDD and the mixture of HCDD tested, the chronic toxicity studies would

be more appropriately used for hazard assessment. The NOEL from the two studies in rats (Kociba et al., 1978, 1979; NTP, 1980a) are 0.001 and 0.0014 μg/kg/day; however, in the mouse (NTP, 1980a), the dose of 0.001 μg/kg/day was a FEL, as indicated by fatty changes in the liver. It would be inappropriate to use a NOEL from rats for hazard assessment when there are data from another species indicating that this dose produces adverse effects.

In addition, it may be inappropriate to derive a toxicity-based hazard assessment for 2,3,7,8-TCDD from these chronic studies, since a three generation study by Murray et al. (1979) indicates that exposure of pregnant rats to this dose of 2,3,7,8-TCDD (0.001 μg/kg/day) throughout gestation resulted in the observation of dilated renal pelvis in the fetuses. Murray et al. (1979) consider this effect not to be treatment-related because it occurred in only one generation at this dose and not at higher doses. Hence, 0.001 μg/kg/day represented a NOAEL. However, a reevaluation of this data by different statistical methods (Nisbet and Paxton, 1980) indicated a statistically significant increase of dilated renal pelvis at higher doses, as well as the lowest one, and lower fetal weight in the 0.001 μg/kg group. With these data, 0.001 μg/kg could be considered a LOAEL. No other studies are available that studied the effects of 2,3,7,8-TCDD at even lower doses.

A toxicity-based hazard assessment is also possible for the mixture of HCDD tested by NTP (1980b). As is shown in Table 14-2, however, the description of the histologic observations was not sufficiently detailed to determine whether the low dose represented a NOAEL or a LOAEL. These data could be used for hazard assessment in either case with an additional uncertainty factor for a LOAEL (45FR79353, 1980).

14.4.2. Animal Carcinogenicity. In addition to the inadequate data base for a toxicity-based hazard assessment, the strong evidence of carcinogenicity in

animals for 2,3,7,8-TCDD would justify a carcinogenicity-based assessment. That two adequate cancer bioassays used sufficiently large groups of animals exposed for an appreciable portion of their lifespan, indicates that 2,3,7,8-TCDD is an animal carcinogen (NTP, 1980a; Kociba et al., 1978) (Table 14-3). In the NTP (1980a) study, male rats developed follicular-cell adenomas or carcinomas of the thyroid.[1] Female rats and mice of both sexes had increased incidences of follicular-cell adenomas of the thyroid.[2] In the study by Kociba et al. (1978), rats maintained on diets that provided doses of 0.0, 0.001, 0.01 and 0.1 µg/kg/day had elevated incidences of carcinomas of the hard palate and tongue, and adenoma of the adrenal cortex in males of the high dose group, and carcinomas of the liver, tongue, lungs in females of the high dose group. The evidence is sufficient to indicate that 2,3,7,8-TCDD is an animal carcinogen.

A single bioassay tested a mixture of the two congeners of HCDD for carcinogenicity (NTP, 1980b). The results summarized in Table 14-4 showed that male and female rats and mice exposed to this mixture of HCDD had increased incidences of neoplastic nodules or carcinomas of the liver. Increased incidence of tumors in two species is sufficient to indicate that this mixture was carcinogenic to animals; however, caution is required in interpreting this data for hazard evaluation since the NTP (1980a) study used a mixture containing two isomers, 1,2,3,6,7,8- and 1,2,3,7,8,9-, of HCDD. There is insufficient evidence to confirm whether both isomers are independently carcinogenic or whether only one isomer or this specific mixture is needed to elicit a carcinogenic response. Since the position of the chlorines may be extremely important for the toxic/carcinogenic properties of HCDD, information obtained from this combined exposure may not be applicable to the individual congeners.

TABLE 14-3

Carcinogenicity Bioassays of 2,3,7,8-TCDD

Exposure Route	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
gavage	rats/ Osborne-Mendel	M	0.0 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	1/69	NTP, 1980a
			0.01 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	5/48	
			0.05 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	8/50	
			0.5 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	11/50	
gavage	rats/ Osborne-Mendel	F	0.0 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	5/75	
			0.1 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	1/49	
			0.05 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	3/50	
			0.5 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	18/49	

TABLE 14-3 (cont.)

Exposure Route	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
gavage	mice/ B6C3F1	M	0.0 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	8/73	NTP, 1980a
			0.01 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	9/49	
			0.05 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	8/49	
			0.5 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	17/50	
gavage	mice/ B6C3F1	F	0.0 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	1/73 0/69	NTP, 1980a
			0.04 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	2/50 3/50	
			0.2 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	2/48 1/47	
			2.0 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	6/47 5/46	

TABLE 14-3 (cont.)

Exposure Route	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
oral	rat/ Sprague-Dawley	F	0.0 µg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma, squamous cell carcinoma of the tongue, squamous cell carcinoma of the lung	0/86 0/86 0/86	
			0.001 µg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma, squamous cell carcinoma of the tongue, squamous cell carcinoma of the lung	0/50 0/50 0/50	
			0.01 µg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma, squamous cell carcinoma of the tongue, squamous cell carcinoma of the lung	2/50 1/50 0/50	
oral	rat/ Sprague-Dawley	F	0.1 µg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma, squamous cell carcinoma of the tongue, squamous cell carcinoma of the lung	11/49 4/49 7/49	Reuber et al., 1978a

TABLE 14-3(cont.)

Exposure Route	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
oral	rat/ Sprague-Dawley	M	0.0 pg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate,	0/85	Kociba et al., 1978a
							squamous cell carcinoma of the tongue,	0/85	
							adenoma of the adrenal cortex	0/85	
			0.001 pg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate,	0/50	
							squamous cell carcinoma of the tongue,	1/50	
							adenoma of the adrenal cortex	0/50	
oral	rat/ Sprague-Dawley	M	0.01 pg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate,	0/50	Kociba et al., 1978a
							squamous cell carcinoma of the tongue,	1/50	
							adenoma of the adrenal cortex	2/50	
			0.1 pg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate,	9/50	
							squamous cell carcinoma of the tongue,	3/50	
							adenoma of the adrenal cortex	5/50	

TABLE 14-A

Carcinogenicity Bioassays of a 1:2 Mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD

Exposure Route	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
gavage	rats/ Osborne-Mendel	M	0.0 µg/kg/week	104 weeks	105 weeks	corn oil acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	0/74	NTP, 1980b
gavage	rats/ Osborne-Mendel	M	1.25 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	0/49	NTP, 1980d
			2.5 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	1/50	
			5.0 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	4/48	
gavage	rats/ Osborne-Mendel	F	0.0 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	5/75	NTP, 1980d
			1.25 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	10/50	
			2.5 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	12/50	
			5.0 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	30/50	

Exposure Route	Species/ Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
gavage/	rats/ Osborne-Mendel	F	0.0 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	15/73	NTP, 1980d
			1.25 µg/kg/week	104 weeks	108 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	14/50	
			2.5 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	14/49	
			5.0 µg/kg/week	104 weeks	108 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	24/48	
gavage	mice/ B6C3F1	F	0.0 µg/kg/week	104 weeks	106 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	3/75	NTP, 1980d
			2.5 µg/kg/week	104 weeks	108 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	4/48	
			5.0 µg/kg/week	104 weeks	108 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	6/47	
			10.0 µg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular adenomas or carcinomas	10/47	