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2,3, 7,8-Tetrachlorodibenzo-p-dioxin

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CHAPTER 8

2,3,7,8-Tetrachlorodibenzo-p-dioxin

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Since 1985, the United States Environmental Protection Agency (USEPA) has classified 2,3,7,8-tetrachloro-p-dioxin (TCDD) as a probable human carcinogen; subsequently, sources of TCDD in the environment have been regulated on the basis of animal cancer rates extrapolated to doses associated with human exposures. Two major activities have prompted the decision to reassess this approach for evaluating TCDD toxicity and its associated risks. First, an epidemiological study of cancer mortality in U.S. chemical workers by the National Institute of Occupational Safety and Health provided evidence of TCDD-mediated human carcinogenicity (Fingerhut et al. 1991). Second, at a 1990 Banbury conference a consensus was reached that TCDD's mode of action involves the activation of a TCDD-receptor complex in susceptible species as a necessary, but not sufficient, prerequisite for any TCDD-related effects (Scheuplein et al. 1991). Based on these findings, USEPA identified a need for biologically based dose-response models for TCDD, and related chemicals, to establish a scientific base for more credible risk assessments. As a consequence, USEPA has designed and implemented a TCDD reassessment research plan. Because of an increasing body of knowledge concerning TCDD exposure to and effects on aquatic life and wildlife, this plan includes a component that addresses ecological risk (USEPA 1993).

An interim analysis of the data and methods that can be used to assess the ecological risk of TCDD was published in 1993 (USEPA 1993). This document critically reviews and evaluates data and models available for analyzing aquatic life- and wildlife-TCDD exposures and effects. Furthermore, it identifies major areas of uncertainty that are likely to limit the degree to which related risks can be characterized. Specifically, the report addresses the direct toxic effects of TCDD and aspects of risk characterization to exemplify approaches and the applicability of available information. This report was subsequently used by a panel of scientists and ecological risk assessors to evaluate data limitations and uncertainties that should be incorporated into future ecological risk assessments of TCDD (USEPA 1994).

The present review draws heavily on the 1993 USEPA report and a recent review by Nosek et al. (1993a) addressing TCDD-related effects on wildlife in terrestrial systems.

Etiology

TCDD is a polychlorinated dibenzodioxin (PCDD) that is chlorinated at the 2, 3, 7, and 8 positions (Fig. 8.1). Compounds within the PCDD family are essentially planar and can be chlorinated at one to eight possible sites. Because of the many possible combinations of chlorination sites in the parent dibenzodioxin structure, there are a large number of PCDD congeners. TCDD is the most toxic member of the PCDDs as well as the toxicologically related polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs).

TCDD is a crystalline solid at ambient temperatures with a melting point of 305°C (Boer et al. 1972). The water solubility of TCDD ranges between 12-20 ng/L in cold water (approx. 4°-12°C) and has been reported between 12.5-19.3 ng/L at 22°C (Marple et al. 1986). The most important physicochemical parameter used to predict and interpret the movement and bioavailability of TCDD in aquatic and terrestrial ecosystems is the octanol/water partition coefficient (K_{ow}). This coefficient is used as a measure of the hydrophobicity of organic chemicals and, in turn, is used to

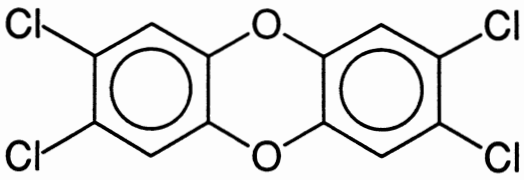

Fig. 8.1. Structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin.

estimate the propensity of a compound to partition into lipid phases of biota or organic carbon in water, sediments, and soils. The K_{ow} is defined as the concentration of a chemical in octanol (C_O) divided by the concentration of the chemical in water (C_W) when the chemical distribution between octanol and water phases is at equilibrium, as represented by the equation: $K_{OW} = C_O/C_W$.

Because of its low water solubility, high hydrophobicity, and the long time periods needed to reach equilibrium between octanol and water, direct measurements of TCDD's K_{OW} are difficult and have required the use of several different techniques. As reviewed by USEPA (1993), the log K_{OW} for TCDD appears to range from approximately 7.0 to 8.0. Thus, at equilibrium, one would expect 10^7 to 10^8 higher concentrations of TCDD in octanol than in water.

Epizootiology

TCDD and other persistent, hydrophobic nonpolar organic chemicals, i.e., chemicals with high log K_{OW} values, partition into organic matter in water and sediments and into lipids in biota. Aqueous concentrations of TCDD in environmental samples are generally not detected because of its low water solubility and partitioning characteristics. Sediments can contain measurable concentrations of TCDD because of its partitioning in waterborne biotic and abiotic solids (USEPA 1993). For example, an average Lake Ontario mean surface sediment concentration of TCDD in 1987 was determined to be 0.068 μg/kg dry sediment (Short et al. 1990). Based on radionuclide-dated sediment core samples, TCDD levels rose from non-detectable before 1940 to a maximum sediment concentration of approximately 0.500 μg/kg dry sediment in about 1962, and then decreased to the 1987 concentrations (USEPA 1993). The sediment core profiles for other PCDDs and PCDFs were similar, but maximum sediment concentrations occurred in different years with different rates of change. A similar sediment concentration pattern was found in the Newark Bay, New Jersey estuary, where surface sediments contained up to 0.730 μg TCDD/kg; however, overlying sediments deposited in the mid-1960s contained 7.6 μg TCDD/kg (Tong et al. 1990). These example sites are associated with past chlorophenol and 2,4,5-trichlorophenoxyacetic acid production. Sediments from many other locations typically have much less TCDD (USEPA 1993).

Fish can be good sentinels for monitoring concentrations of TCDD in aquatic systems. At log K_{ow}s greater than 5.0, exposure through ingestion of contaminated food becomes an important route for uptake in aquatic life (Thomann 1989). Thus, all PCDDs, PCDFs, and PCBs that elicit a TCDD mode of toxic action are significantly accumulated in fish through food ingestion. Exposure differences across ecosystems can occur as a result of temporal and spatial variation of the distribution of the chemical between the water, sediment, and food that organisms contact (USEPA 1993).

Several surveys undertaken by USEPA provide data regarding the distribution of TCDD residues in fish. The USEPA National Dioxin Survey was an evaluation of TCDD residues in fish from 395 sites in the United States (Kuehl et al. 1989; USEPA 1987). TCDD concentrations in fish ranged from below detection at 0.001 μg TCDD/kg wet weight of whole organism (72% of all samples) to a maximum concentration of 0.085 μg/kg. TCDD was not detected in fish samples taken from 73 of 90 randomly selected sites across the United States, but it was detected in 23 of 29 Great Lakes sites (USEPA 1987). In general, highest concentrations among all fish sampled in the survey were associated with the open Great Lakes and river sites downstream from kraft paper mills. In the 29 sites sampled in the Great Lakes, TCDD exceeded 0.005 μg/kg in 60% of the samples. Consistent with the decline in contaminant loading and sediment concentrations, TCDD residues in stocked lake trout, *Salvelinus namaycush,* in Lake Ontario have decreased to approximately 25% of the levels present in 1977 (USEPA 1993). In another study, TCDD was detected in fish from 70% of sites sampled across the United States in 1986-1988; the maximum TCDD concentration was 0.204 μg/kg, with an average of 0.0068 μg/kg (USEPA 1992).

It should be pointed out that the sampling sites in these USEPA surveys were not random samples of U.S. waters, but were skewed towards waters affected by anthropogenic activities. Also, there are locations not included in these surveys that have much higher contamination levels (USEPA 1993). For example, striped bass, *Morone saxatilus,* from

the lower Hudson River and its estuary were found to contain TCDD concentrations up to 0.120 μg/kg in comparison to 0.001 to 0.004 μg/kg in striped bass from Chesapeake Bay (O'Keefe et al. 1984).

Invertebrate species also have been used to monitor distributions of PCDDs, PCDFs, and PCBs in aquatic ecosystems. For example, the mussel, *Elliptio complanata,* has been used to assess TCDD accumulation from water associated with pulp and paper mills and other sources in the Rainy River watershed of Ontario, Canada. Mussels placed at this site accumulated up to 0.01 μg TCDD/kg after 21 d (Hayton et al. 1990).

Amphibian species have been given only scant attention in regard to studies of TCDD accumulation. Adult bullfrogs, *Rana catesbeiana,* collected in 1984-1985 near a former 2,4,5-trichlorophenoxyacetic acid production site along Rockey Branch Creek in Arkansas, contained 0.640-48 μg TCDD/kg of liver (Korfmacher et al. 1986a). Bullfrog muscle tissue samples contained less than 10% of the liver TCDD concentrations (Korfmacher et al. 1986b).

Monitoring TCDD levels in the eggs of wild birds also has been useful in assessing exposures over time and across habitats. For example, during the 1970s and 1980s TCDD levels were monitored in the eggs of herring gulls, *Larus argentatus,* that nested on several islands in Lake Ontario (Environment Canada 1991a). In 1971 and 1972, TCDD levels of approximately 2.0 to 2.4 μg/kg were reported for gull eggs taken from nesting sites on Scotch Bonnet Island; however, in 1977 and 1978 levels were down to 0.500 μg/kg, and in 1982, levels had further decreased to 0.204 μg/kg. Concentrations of TCDD in gull eggs taken from nests on Snake Island dropped from approximately 0.175 μg/kg in 1981 to 0.090 μg/kg in 1989. At Muggs Island, TCDD concentrations have remained relatively constant at approximately 0.025 to 0.05 μg/kg in gull eggs examined from 1984 through 1989. The reduction of TCDD levels in gull eggs is consistent with discontinued TCDD inputs from waste sites and chemical manufacturing plants and associated decreases in water, sediment, and fish concentrations.

In similar studies, Braune and Norstrom (1989) examined the relationship between TCDD accumulation in herring gulls and alewife, *Alosa pseudoharengus,* in Lake Ontario. In 1985, TCDD concentrations were reported to be 0.127 μg/kg whole body weight in herring gull and 83 μg/kg in herring gull eggs, while alewife concentrations were reported to be 0.004 μg/kg. Biomagnification factors between whole-body herring gull and alewife, and herring gull egg and alewife, were subsequently calculated to be 32 and 21, respectively.

Marine mammals have attracted the interest of many researchers who are concerned with the accumulation of PCDDs, PCDFs, and coplanar PCB congeners. Typically, coplanar PCB congeners have been reported to accumulate in marine mammals to a much greater degree than TCDD. For example, in the 1980s concentrations of coplanar PCBs in Baird's beaked whale, *Berardius bairdi,* killer whale, *Orcinus orca,* and finless porpoise, *Neophocoena phocoenoides,* ranged from approximately 20 to 65,000 μg/kg, while TCDD concentrations ranged from less than 0.0005 to less than 0.001 μg/kg (Kannan et al. 1989).

Recently, Nosek et al. (1993a) reviewed and summarized several studies that addressed the accumulation of TCDD in terrestrial mammals at a contaminated field site in Florida. In terrestrial ecosystems in which the soil has been contaminated with TCDD, the burrowing mammals are the species most generally associated with high residues. For example, mice, *Peromyscus polionotus,* and hispid cotton rats, *Sigmodon hispidus,* exposed to soil contaminated at levels as high as 1.5 μg TCDD/kg had liver concentrations up to 2.9 and 0.210 μg TCDD/kg, respectively. Conversely, although burrowing mammals showed high residues, TCDD was not detected in white-tailed deer, *Odocoileus virginianus,* Virginia opossums, *Didelphis marsupialis,* or eastern cottontail rabbits, *Sylvilagus floridanus.*

Pathogenesis

TCDD is the most potent member of a variety of structurally similar PCDDs, PCDFs, and several non- and mono-ortho-substituted (planar) PCBs that appear to act via the same mode of action (Poland and Knutson 1982; Safe 1990; Whitlock 1990). The initial step by which TCDD and related compounds are thought to exert their toxicity is through binding to the cytosolic aryl hydrocarbon (Ah) receptor (Poland and Glover 1980), followed by translocation of the ligand-receptor complex to the nuclear DNA. Resulting transcription of one or more target genes (Denison et al. 1989; Nebert 1990; Whitlock 1990) is postulated to ultimately result in widely varying responses.

The physiological effects observed during TCDD intoxication are reasonably consistent across vertebrates. Characteristic of TCDD-induced toxicity is a delayed onset of death, even at relatively large doses. Additional effects include weight loss ("wasting syndrome"); decreased immunocompetence; subcutaneous edema; reproductive effects (fetotoxicity, teratogenesis); alterations in lipid metabolism and gluconeogenesis; thymic atrophy; and

induction of cytochrome P4501A1, among other enzyme systems (Poland and Knutson 1982; Safe 1990). The doses at which these effects are elicited, however, vary significantly. For example, in the guinea pig LD_{50} and ED_{50} values for lethality, body weight loss, and hepatic microsomal monooxygenase induction are 0.600 to 2.0, 1.4 and 0.070 μg/kg, respectively (Safe 1990).

Interspecies differences to TCDD intoxication can be quite large and must be acknowledged in assessing risk. Because little or no toxicological data are available for specific wildlife species of concern, the need to extrapolate toxic responses from surrogate species is required. Species-specific toxicokinetic factors, such as uptake, disposition, metabolism, and elimination of TCDD, as well as differences in concentration, tissue distribution, and ligand affinity of the Ah receptor, contribute to the variable interspecies responses to TCDD exposure. The presence of the Ah receptor, however, appears to be necessary for TCDD, and related compounds, to exhibit the specific toxic responses discussed previously. In addition, the relative species-specific affinity of the Ah ligand for TCDD-like compounds is correlated to their relative toxic potency across species (Bandiera et al. 1984; Mason et al. 1986; Poland and Glover 1977). Thus far, detectable concentrations of the Ah receptor across a number of different tissues have been reported for all mammals and birds studied. This suggests that it is reasonable to assume that since standard laboratory species have the Ah receptor, it also is present in avian and mammalian wildlife that have not yet been tested. The Ah receptor also has been conclusively demonstrated in teleost and elasmobranch fishes (Hahn et al. 1992; Lorenzen and Okey 1990) but has not been detected in some primitive fishes, e.g., hagfish and lampreys, and has not been found in nine species of invertebrates representing eight classes of four phyla (Hahn et al. 1992). The presence of the Ah receptor in amphibians and reptiles remains uncertain, as relatively sensitive detection techniques have not been applied to these animal classes (USEPA 1993).

One study has been reported that examined the toxic effects of TCDD on amphibians. Beatty et al. (1976) injected tadpoles and adult bullfrogs intraperitoneally with TCDD doses of 25-1,000 and 50-500 μg/kg body weight, respectively. Through 50 d post-dose, no tadpole deaths were attributed to TCDD and all surviving tadpoles were observed to successfully complete metamorphosis. No adult bullfrogs died in any of the treatment groups through 35 d post-injection. Although food intake was initially lessened in animals exposed to the highest dose of 500 μg/kg, food consumption was not different from that of the controls at the completion of the study.

Studies by Aulerich et al. (1988) and Hochstein et al. (1988) provide information on the toxicity of TCDD to mink, *Mustela vison,* following exposures of 1 to 12 d. Hochstein et al. (1988) administered TCDD as a single oral dose to adult male mink and reported a 28-d LD_{50} of 4.2 μg/kg. Aulerich et al. (1988) administered doses of 0.1 and 1.0 μg/kg TCDD by intraperitoneal injection to newborn mink for 12 consecutive days. Newborn mink exposed to TCDD at a dose of 1.0 μg/kg died within 14 d, and 62% of the mink exposed to 0.1 μg/kg died within 19 wk.

Mink are among the mammalian species most sensitive to TCDD intoxication (Aulerich et al. 1988; Hochstein et al. 1988). Based on a 28-d LD_{50} of 4.2 μg/kg (Hochstein et al. 1988), mink seem to be less sensitive than guinea pigs, for which LD_{50}s of 0.6 to 2.0 μg/kg have been reported, but more sensitive than rats (LD_{50}s of 22 to 45 μg/kg); rabbits (LD_{50} of 115 μg/kg); mice (LD_{50}s of 114 to 284 μg/kg); and hamsters (LD_{50}s of 1,157 to 5,000 μg/kg) (Kociba and Schwetz 1982a, b; Schwetz et al. 1973).

Several studies have been undertaken to determine the lethal potency of TCDD to avian wildlife. Hudson et al. (1984) reported 37-d LD_{50}s of 15 for the bobwhite quail, *Colinus virginianus*; > 108 for the mallard, *Anas platyrhynchos,* and > 810 μg/kg for the ringed turtle dove, *Streptopelia risoria,* following a single oral administration of TCDD. By comparison, Grieg et al. (1973) reported that chickens, *Gallus gallus,* given single oral doses of TCDD at 25 to 50 μg/kg died within 12 to 21 d post-treatment. In a study carried out over a longer period of time, Schwetz et al. (1973) orally administered TCDD to 3-day-old white leghorn chickens for 21 d and reported a no observable adverse effect level (NOAEL) for mortality of 0.1 μg/kg/d with the lowest observable effect level (LOAEL) at 1 μg/kg/d. Nosek et al. (1992a) injected ring-necked pheasants, *Phasianus colchicus,* intraperitoneally with single doses of TCDD and observed the birds for 11 wk post-treatment. All birds treated at the high dose of 100 μg/kg died within 6 wk of exposure. Birds exposed to 25 μg/kg began to die 6 wk after treatment, and at 11 wk post-exposure, 80% were dead. No birds died in the 6.25 μg/kg exposure group during the course of the study. Common to all of the above mentioned studies, a dose-dependent decrease in food consumption and body weight preceded death.

Reproduction

Studies describing the effects of TCDD on the reproductive or developmental aspects of mammalian wildlife are not available in the literature.

However, several studies have been reported that involve typical laboratory mammals (Peterson et al. 1993). Studies by Murray et al. (1979) with Sprague-Dawley rats and by Bowman et al. (1989a, b) with Rhesus monkeys, *Macaca mulatta,* serve to illustrate how organismal-level responses in laboratory species may be applicable for projecting population-level mammalian-wildlife risk assessments (USEPA 1993).

Functional alterations in development associated with the toxicity of TCDD in traditional laboratory mammals, appear to be more sensitive than endpoints of male or female reproductive toxicity (Peterson et al. 1993). For example, in the rat, functional alterations in the male reproductive system, including reduction in spermatogenesis and changes in masculine sexual behavior in adulthood, have been reported at maternal doses below those that cause overt toxicity to the dams or offspring. Effects on male reproductive development may be due to the ability of TCDD to decrease androgenic plasma concentrations during early development. These developmental responses may be noteworthy in considering responses in mammalian wildlife populations; however, as with reproductive effects, interspecies variability must be considered.

Although there are no reproductive or developmental studies reported for mammalian wildlife species, there are studies in the literature for avian species. Nosek et al. (1992a) investigated the effects of TCDD on reproduction in ring-necked pheasants. Hens were administered TCDD by intraperitoneal injection once a week for 10 wk and during the final 2 wk of exposure, were paired with roosters and kept in egg production for an additional 9 to 13 wk. A 57% death rate occurred in hens administered TCDD at 1 μg/kg/wk. A significant decrease in adult body weight and egg production also was associated with this dose level. There was a trend toward increased embryo deaths as the dose to the hens was increased. A dose of 0.450 μg/kg/wk was calculated to elicit a 50% increase in embryo mortality above the control rate.

Nosek et al. (1993b) also injected fertile ring-necked pheasant eggs with graded TCDD doses of 0.00001 to 100 μg/kg. The number of chicks that died was noted through 28 d post-hatch. A dose-dependent increase in embryo mortality was observed following both albumin and yolk injections, and LD_{50}s of 1.4 and 2.1 μg/kg, respectively, were reported. Based on injections into the yolk, the LOAEL for mortality was 10 μg/kg, and the NOAEL was 1.0 μg/kg. Results from albumin injection studies indicated a LOAEL of 1.0 μg/kg and a NOAEL of 0.1 μg/kg. Martin et al. (1989), as cited by Nosek et al. (1993b), reported 100% embryo mortality in Eastern bluebirds, *Sialia sialis,* following injection into the albumin of TCDD at a dose of 10 μg/kg and no mortality at 1.0 μg/kg.

Based on in ovo exposures, the chicken may be more sensitive to TCDD than the pheasant or bluebird. Henshel et al. (1993) reported a NOAEL of 0.1 μg/kg following injections into the yolk of fertile eggs. Cheung et al. (1981) reported up to 30% mortality in chicken eggs injected at 0.00005 to 0.450 μg/kg (assuming an egg weight of 0.055 kg) in the albumin; however, a significant linear log dose-response relationship was not observed. Following injection into the airspace of fertile chicken eggs, an LD_{50} value of approximately 0.250 μg/kg has been reported (Allred and Strange 1977).

Several field studies provide insights regarding the relationship between TCDD exposure and reproductive success in birds. The productivity of herring gull colonies at Snake, Muggs, and Scotch Bonnet Islands in Lake Ontario was monitored from 1972 through 1984 (Environment Canada 1991b; Mineau et al. 1984). From 1971 through 1975, productivity (defined as the number of young reaching 21 d of age per nesting adult) ranged from 0.06 to 0.21, which was well below the range of 0.8 to 1.0 required for population stability. However, in 1977 productivity values exceeded 0.8 and through 1984 (the last year of reported data) ranged from 0.86 to 2.13. The population of gulls increased from 520 pairs in 10 colonies in 1976 to 1,540 pairs in 15 colonies in 1987. During the period of poor reproductive performance, a suite of adverse effects that are commonly associated with TCDD and toxicologically related compounds (Gilbertson et al. 1991) were reported. However, eggshell thinning of only 4% to 8% was noted in this DDE-resistant species, a factor that strongly suggested total DDT exposure was not associated with the low productivity (Environment Canada 1991b).

During the time that herring gull productivity was assessed (Environment Canada 1991b), TCDD residues in eggs also were monitored (Environmental Canada 1991a). In 1971 and 1972, TCDD levels of approximately 2.0 to 2.4 μg/kg in gull eggs were reported for Scotch Bonnet Island (1972 productivity of 0.12); in 1974, levels were down to approximately 0.9 μg/kg (1973 and 1975 productivities of 0.06 and 0.15); and in 1977 and 1978, they were at 0.5 μg/kg (productivities of 1.10 and 1.01). In 1982 levels were down to 0.204 μg/kg (1981 productivity of 2.13). Concentrations of TCDD in gull eggs from Snake Island dropped from approximately 0.175 μg/kg in 1981 (productivity of 1.73) to 0.09 μg/kg in 1989. At Muggs Island, TCDD concentrations in gull eggs have remained relatively constant at approximately 0.025 to 0.05 μg/kg from 1984 through 1989 (with prior 1981 and 1984 productivities of 1.40 and 1.17).

In studies by Bellward et al. (1990) and Hart et al. (1991), survival and growth of great blue heron, *Ardea herodias,* chicks were monitored in colonies from three sites in British Columbia that varied in PCDF and PCDD concentrations. The predominant congeners present in great blue heron eggs from these sites were TCDD 1,2,3,7,8-pentachlorodibenzo-p-dioxin and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin. Mean TCDD concentrations (± SEM) in eggs from these sites were 0.01 ± 0.0009, 0.135 ± 0.0496, and 0.211 ± 0.0337 μg/kg. Hatching success did not differ significantly across sites but there was an apparent increase in the incidence of edema in chicks with increasing TCDD concentration. In addition, there was an inverse correlation between TCDD egg concentrations and various growth measurements made on the heron chicks. In a more recent study, preliminary results suggest there also may be an inverse association between TCDD egg concentrations and morphometric changes in the brains of these hatchlings (Henshel et al. 1992).

Interactions with Other Chemicals

As discussed above, the planar PCBs and certain substituted PCDFs and PCDDs, including TCDD, appear to act via the same mode of toxic action. This suggests that in mixtures of PCBs, PCDFs, and PCDDs these compounds could interact in an antagonistic, synergistic, strictly additive or nonadditive manner. Of particular concern is the potential for additivity that has been documented for certain endpoints (Birnbaum et al. 1987; Eadon et al. 1986; Pluess et al. 1988; Sawyer and Safe 1985; Vecchi et al. 1985; Weber et al. 1985). Thus, the combination of these compounds in a sample could result in toxicity, even though an individual PCB, PCDF, or PCDD congener may not be present at a toxic concentration. The potential toxicity of mixtures of PCBs, PCDFs, and PCDDs in environmental samples has been evaluated using two basic approaches to express potency relative to TCDD. In one approach concentrations of individual PCB, PCDF, and PCDD congeners in an environmental mixture are quantified and multiplied by toxic equivalent factors (TEFs), which then are summed to express potential toxicity in TCDD-equivalents. Bellward et al. (1990) and White and Seginak (1994), provide examples of how this approach has been applied to wildlife assessments. The TEFs are derived from in vitro or in vivo studies evaluating the potency of individual congeners relative to TCDD; however, it should be noted that many of the TEFs currently employed for wildlife risk assessments are derived from induction of cytochrome P4501A1 in laboratory mammals or mammalian cell lines. Questions remain as to the exact relationship between P450 induction potency and in vivo toxicity of PCBs, PCDFs, and PCDDs, the influence of exposure length on congener-specific in vivo potency (De Vito et al. 1993) and the extrapolations from mammalian systems to nonmammalian species. With regard to the latter issue, development of TEFs based on salmonid sac fry mortality are noteworthy (Walker and Peterson 1991).

In the second method for determining sample TCDD-equivalents, total PCB/PCDF/PCDD-mixture extracts from environmental samples are tested for potency, relative to TCDD. Typically, induction of cytochrome P4501A1, and associated monooxygenase activities, in the H4IIE rat hepatoma cell line is used. This technique has the added advantage of incorporating potential antagonistic or synergistic interactions between PCDD, PCDF, and PCB congeners. This approach has been used in a number of instances to evaluate the potential impact of environmental PCB, PCDF, and PCDD mixtures to avian wildlife (Tillitt et al. 1991, 1992). As with the first method, biological systems and responses other than enzyme induction in a mammalian cell line may be needed for measuring the overall toxicity response and quantifying extrapolations to endpoints and species appropriate for wildlife risk assessments.

Interactions with Infectious Agents

There also has been increased concern over the role played by TCDD and related compounds in suppressing the immune system and the consequent increase in vulnerability to infectious agents (Holsapple et al. 1991). In rodents, immunosuppression and increased susceptibility to infection are among the more sensitive TCDD toxic responses. However, there may be significant differences between rats and mice, suggesting that extrapolations of these responses to mammalian wildlife may not be straightforward. For example, Yang et al. (1994) reported that doses between 0.1 and 0.01 μg TCDD/kg increased mortality due to influenza virus in mice, while in the rat, immunotoxicity (expressed as increased viral titer and suppression of virus-augmented natural killer cell activity) was observed at 10 and 3.0 μg TCDD/kg.

A limited number of studies have been reported that are concerned with immunotoxic responses in wildlife. In the same egg injection study outlined previously, Nosek et al. (1993b) assessed immune response in pheasant chicks through 28 d post-hatch. Injections of TCDD into the albumin at 0.001, 0.01, 0.1 or 1.0 μg/kg had no effect on immune response, as monitored by serum titers of

IgG, IgM, and total antibody in 28-day-old chicks injected with washed sheep erythrocytes.

Pathology

In adult male mink exposed to single oral doses of 5.0 and 7.5 μg TCDD/kg, gross necropsy revealed mottling and discoloration of the liver, spleen, and kidneys, concomitant with enlargement of the brain, kidneys, heart, and thyroid and adrenal glands. Animals that survived a TCDD exposure at 2.5 μg TCDD/kg showed no alterations in hematological and thyroid hormone measurements (Hochstein et al. 1988).

TCDD and related PCDDs, PCDFs, and PCBs produce a syndrome of edema and histological alterations in chickens (Gilbertson et al. 1991). For example, investigators have reported edema, involution of the bursa of Fabricius, and cardiovascular malformations in chickens following intraperitoneal, dietary or in ovo exposure, respectively, to TCDD (Cheung et al. 1981; Flick et al. 1972; Sawyer et al. 1986). Cheung et al. (1981) reported concentrations of approximately 0.0058 μg/kg egg (assuming an egg weight of 0.055 kg) associated with the combined occurrence of four cardiovascular malformations (ventricular septal defect, aortic arch anomaly, aortic arch anomaly plus ventricular septal defect, and conotruncal malformation) in 50% of chicken embryos. There was approximately a 30% incidence rate for combined malformations in control groups, for which a similar rate was observed in uninjected, sham-injected, and vehicle-injected eggs. There were, however, no significant relationships between TCDD exposure and the percentage of embryos having cardiac malformations when each of the four defects was analyzed separately. Henshel et al. (1993) reported abnormalities in chicken embryos through 4 d of incubation that included asymmetrical somites, heart malformations, malformed visceral arches, decreased vitelline vasculature, and variations in the chronological expression of various developmental indicators. Of the abnormalities noted, a decrease in area covered by vascularized blastoderm was most clearly related to TCDD concentration (Henshel et al. 1993).

At nonlethal in ovo doses, Nosek et al. (1993b) and Martin et al. (1989) reported that there was no evidence of TCDD-related effects on chick growth, histopathological abnormalities, or the incidence of edema, ascites, or hydropericardium in pheasants or Eastern bluebirds. Also, there was no significant TCDD effect on histology of the liver, spleen, heart, bursa of Fabricius, or thymus. These differences between the chicken, pheasant and bluebird might reflect variations in routes of exposure and/or species sensitivity.

Beatty et al. (1976) injected tadpoles and adult bullfrogs intraperitoneally with TCDD at doses of 25-1,000 and 50-500 μg/kg body weight, respectively. Through 50 d post-dose, histopathological examination of the liver, heart, kidney, lung, and reproductive organs in tadpoles, done shortly after the completion of metamorphosis, revealed no lesions. Histopathological examination of several organs in adult bullfrogs revealed no significant lesions at any of the TCDD doses administered.

Diagnosis

An analysis of tissue residues is an essential component of ecological risk assessment and water quality criteria development for TCDD and related chemicals because long-term chemical accumulation in tissues of the organisms of concern, or their food source, is the best method for evaluating toxicity (USEPA 1993). Currently, high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is used to provide the most sensitive and reliable measurements of TCDD concentrations in environmental samples. Minimum levels of TCDD detection for HRGC/HRMS have been reported to be 0.0005 μg/kg, 0.001 μg/kg, 0.005 ng/L, and 0.0005 ng/L for tissue, sediments, water-solids, and water, respectively (USEPA 1993).

When TCDD contamination can be confirmed in biota within an ecosystem, it is necessary to relate these residue levels to toxicological information to assess whether or not there is sufficient TCDD exposure to cause adverse effects. The approach used here to express wildlife-effects profiles for species in aquatic ecosystems is similar to that proposed by USEPA (1993), the Great Lakes Water Quality Initiative procedure for deriving criteria for the protection of wildlife (USEPA 1991), and an analysis of risk of selenium to wildlife (Peterson and Nebeker 1992). These efforts were intended to identify the highest dietary or aqueous concentrations of toxicants that would not cause unacceptable reduction in the growth, reproduction, or viability of representative mammalian and avian species that ingest surface water or aquatic life taken from surface waters. Because the concentration of TCDD in food organisms, e.g., fish, is generally 10^5 times or greater than that of the water, food consumption provides nearly all TCDD exposure.

For the analyses summarized here, the following equation can be used to estimate food concentrations associated with an adverse effect level in wildlife (USEPA 1993):

$$FC = EL/EF_S \times EF_C \times Wt_A/F_A = EL/EF_S \times EF_C \times R_A$$

where:

- FC = Food concentration associated with an effect level (μg/kg)
- EL = Effect level from a toxicity study (μg/kg/d)
- Wt_A = Weight of the organism of interest (kg)
- EF_S = Extrapolation factor for relative species sensitivity
- EF_C = Extrapolation factor for subchronic to chronic exposure
- F_A = Food consumption for the organism of interest (kg/d)
- R_A = Food consumption as fraction of weight (L/d)

The analyses discussed below use this equation and consider risk to mammalian and avian wildlife species that have a diet consisting solely of fish, or other aquatic macrofauna that would have TCDD and caloric-content levels comparable to fish. Representative mammalian wildlife species would include the river otter, *Lutra canadensis,* and mink, and representative avian wildlife would include bald eagle, *Haliaeetus leucocephalus*; osprey, *Pandion haliaetus*; kingfishers; terns; herons; diving ducks; mergansers; and loons, *Gavia* sp. For mink and river otter, the food consumption rate (R_A) would be expected to be in the range of 10%-20% of body weight per day (Aulerich et al. 1973; Bleavins and Aulerich 1981; Lauhachinda 1978; Linscombe et al. 1982; Newell et al. 1987; Toweill and Tabor 1982). For birds, the value can range from 10%–50% (Alexander 1977; Bortolotti 1984; Craig et al. 1988; Fry 1980; Nagy 1987; Newell et al. 1987; Palmer 1988; Poole 1989; Stalmaster and Gessaman 1982, 1984).

Analyses using the equation assume a diet that is completely piscivorous. However, other dietary sources can alter the presumed risk of derived fish tissue concentrations, e.g., the results of this approach would overestimate the risk to mink that are not primarily foraging for fish and aquatic invertebrates but feeding instead on terrestrial organisms. Conversely, for bald eagles that consume fish-eating birds (Kozie and Anderson 1991), which biomagnify TCDD (Braune and Norstrom 1989), analyses based on the equation would underestimate exposure. Of course, the equation can be modified to incorporate more refined dietary assumptions.

To establish an effects profile for mammalian wildlife based on reproductive effects, a study by Murray et al. (1979) with Sprague-Dawley rats is most relevant for assessing adverse effects in wildlife populations because it incorporated an exposure regime over three generations. The results of this study indicate a NOAEL of 0.001 μg/kg/d for reproductive capacity of rats, i.e., fertility, litter size, gestational survival, and neonatal survival and growth. However, as discussed previously, mink appear to be one of the mammals most sensitive to TCDD intoxication and based on a comparison of LD_{50} values, is approximately one order of magnitude more sensitive than the rat. Using the equation, the analysis suggests that TCDD poses no demonstrable risk to mammalian wildlife if daily intake does not exceed 0.0001 μg/kg/d, based on a rat NOAEL of 0.001 μg/kg/d and an interspecies extrapolation factor (EF_S) of 10. Applying an interspecies extrapolation factor (EF_S) of 10 to the rat NOAEL results in a value roughly equivalent to using a NOAEL of 0.00013 μg/kg/d for a reproductive success, i.e., pregnancy rate, abortion rate, stillbirth rate, and survival through 1 yr, in rhesus monkeys with no interspecies extrapolation factor (Bowman et al. 1989a, b). For a consumption rate (R_A) of 10-20%, this daily intake corresponds to concentrations in fish of 0.0005-0.001 μg/kg. For sensitive organisms, substantial effects on reproduction would be expected at concentrations approximately 10-fold higher.

Based on an analysis of the reproductive effects of TCDD in pheasant (Nosek et al. 1992a) compared to the sensitivity of other birds, and on toxicokinetic considerations of the pheasant reproduction study (Nosek et al. 1992b), an exposure associated with low risk to avian wildlife of 0.0014 μg/kg/d can be calculated. This value is based on a NOAEL of 0.014 μg/kg/d, an EF_S of 1 and EF_C of 10. For a food consumption rate of 10-50% of body weight per day, this daily intake would correspond to a TCDD concentration in fish of 0.003 to 0.0014 μg/kg. Substantial effects on the reproduction of sensitive birds would be expected at chronic exposures approximately 10 times higher.

The data for herring gulls and great blue heron given previously indicate that successful reproduction does occur in wild colonies, i.e., maintenance of a stable population, even when TCDD is present in eggs at concentrations from between 0.2 to 0.5 μg/kg. In turn, toxic effects at the individual level are associated with concentrations above 0.1 μg/kg. Braune and Norstrom (1989) reported a biomagnification factor of 21 for TCDD in herring gull eggs compared to alewife in Lake Ontario; thus, the egg concentrations associated with a NOAEL for population effects (0.2 to 0.5 μg/kg) are approximately equivalent to 0.01 to 0.024 μg TCDD/kg

forage fish. Forage fish concentrations of approximately 0.005 μg/kg would be associated with an egg mortality NOAEL of 0.10 μg/kg egg. These derived fish tissue concentrations should not be assumed appropriate for all aquatic ecosystems because they are based on a Lake Ontario–specific biomagnification factor and bioaccumulation is a site-specific process (USEPA 1993). It also should be noted that the wild populations from which these analyses are derived are subject to a variety of other stressors, including other hydrophobic chemicals (PCDDs, PCDFs and PCBs), that could act jointly with TCDD. As a consequence, this field-derived TCDD NOAEL is somewhat reflective of chemical mixtures in Lake Ontario. Therefore, if TCDD were the only toxicant present in the Lake Ontario ecosystem, the NOAEL would presumably be higher than 0.01 to 0.024 μg TCDD/kg.

Nosek et al. (1993a) have developed a model to assess the potential impact of contaminated terrestrial dietary sources, e.g., insects, seeds, soil, on the ring-necked pheasant. This model provides a calculation of a predicted steady-state TCDD concentration in the ring-necked pheasant that can be compared to NOAELs or LOAELs. The model is based on an allocation of ring-necked pheasant food sources by mass, TCDD bioavailability in different food sources, and a half-life estimate for elimination of TCDD in the pheasant (378 d) (Nosek et al. 1992b). Using this model, an estimated steady-state TCDD concentration in ring-necked pheasant of 1 μg/kg wet weight, which is equivalent to the NOAEL for reproductive effects (Nosek et al. 1992a) expressed as an accumulated dose, would be equated to soil, earthworm and insect concentrations of approximately 0.5, 1.67, and 0.29 μg TCDD/kg wet weight, respectively. This estimate of dietary NOAEL concentrations assumes that pheasants ingest approximately 0.083 kg of uncontaminated seeds, 0.004 kg of contaminated insects (TCDD bioavailability of 58%), 0.0013 kg of contaminated earthworms (bioavailability of 30%), and 0.004 kg of contaminated soil (bioavailability of 33%) per day, and that the relative ratio of TCDD in earthworms and insects to soil is similar to that observed in an industrial red pine, *Pinus resinosa,* plantation where measured soil, earthworm and cricket TCDD concentrations were reported to be 0.0108, 0.036, and 0.0067 μg/kg wet weight, respectively (Nosek et al. 1993a). This analysis is specific for the ring-necked pheasant, and extrapolations to other terrestrial species would require estimates of species-specific TCDD toxicokinetic data and NOAELs, as well as appropriate dietary source information.

Treatment, Control, and Prevention

TCDD, and other PCDDs and PCDFs, are not commercial products, but rather unintended by-products typically associated with chlorophenol and chlorophenoxyacetic acid production, chlorine bleaching in paper mills, and numerous combustion processes, e.g., industrial and municipal incinerators, residential heating, that involve phenolic organic materials and chlorine (Safe 1991). Because of TCDD's extreme toxicity and its accumulation in fish and other human food sources, releases of TCDD into the environment have been markedly reduced over the last 15 to 20 yr. As discussed above, there is evidence to indicate that for the Great Lakes, reduced inputs have led to decreasing concentrations of TCDD and related compounds in sediments and biota. Extensive research efforts are in progress throughout the world to better understand and quantify the toxic effects of TCDD and related chemicals, as well as their fate and transport. In turn, these research efforts are contributing to improved techniques for assessing the impact, controlling releases, and tracing the environmental movement of this class of compounds.

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