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# Long-Term Hazards of Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans*

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**During January 10–11, 1978 in Lyon, France, a joint National Institute of Environmental Health Sciences/International Agency for Research on Cancer *ad hoc* Working Group considered and discussed the feasibility of coordinating epidemiological studies on the long-term hazards associated with the chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans (PCDDs and PCDFs). Nineteen invited scientists from eight countries presented introductory working papers summarizing the most up-to-date and relevant information available from their individual programs. This report represents the collective views and scientific opinions of the Working Group. The greater part of this document comprises epidemiological studies related to episodes of human exposure. The review begins with a brief section concerning possible routes of human exposure, an overview of the pertinent chemical characteristics, and the salient toxicological properties of the structurally siimilar PCDDs/PCDFs. The Working Group report ends with recommendations for future activities.**

## Introduction

Human exposure in the workplace can occur when chlorinated dibenzo-*p*-dioxins (PCDDs) are formed during the production of certain compounds such as the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), the fungicide pentachlorophenol, and the germicide hexachlorophene (*1*). The dioxins, impurities/contaminants associated with these end-products, result most often from treatment of chlorinated benzenes at elevated temperature and pressure under alkaline conditions. Via the widespread use of these commercial products (*1*, *2*) the general population may also become exposed.

In recent years, out-of-control chemical reactions during the production of 2,4,5-trichlorophenol have proceeded to the explosive

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stage thereby exposing persons to toxic levels of PCDDs. From certain of these accidents, moreover, not only did those occupationally involved receive dangerous exposure but also those inhabiting the surrounding areas received uncommon risk.

The structurally-related chlorinated dibenzofurans (PCDFs) are contaminants found in some polychlorinated biphenyl compounds: Aroclor, Clophen, Phenoclor (*3*).

These impurities are more toxic and represent a greater environmental hazard than the compounds they contaminate (*1, 3-9*). For instance, 2,3,7,8-tetra-CDD has been recently found in beef fat from cattle grazed on 2,4,5-T-treated rangeland and in breast milk from women living in areas where 2,4,5-T is used on rangeland or in forest areas (*10*). Various investigators (*11-14*) have also discovered PCDDs and PCDFs in fly ash and flue gas from municipal incinerators, and in dust from fungicide-treated wood (*15*).

## Chemical Aspects

The PCDDs and PCDFs are two series of tricyclic aromatic compounds which exhibit similar chemical and physical properties (*1, 3, 4*). The basic two-dimensional structures (I, II) have eight possible points of chemical addition. From the monochloro to the octachloro derivatives, a variety of positional isomers are possible: 75 PCDDs and 135 PCDFs (Table 1).

The extreme toxic potency of some of these compounds, as well as the large number of potential isomers, warrant analytical methods exhibiting high sensitivity and specificity to monitor the environment. A desirable detection limit of one part per trillion (ppt, 1 picogram/gram sample) is being reached with current methodology. Essential requirements included efficient clean-up, good separation and selectivity, ultrasensitive quantification, and validation (*16, 17*).

## Toxicological Aspects

### Animals

The prototype and most extensively studied isomer of the PCDDs and PCDFs is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-tetra-CDD), perhaps the most potent man-made toxin presently known. The comparative oral lethal dose values for various PCDDs in mice and guinea pigs (Table 2) show clearly that the 2,3,7,8-tetra-CDD and the 1,2,3,7,8-penta-CDD isomers are the most toxic (*18*).

Toxic effects induced by PCDDs and PCDFs vary quantitatively and qualitatively among different species; however, within a single species the untoward consequences are markedly similar for all PCDDs and PCDFs that have been studied. For example, the toxic effects induced by 2,3,7,8-tetra-CDD which are most often observed in mice, guinea-pigs, and monkeys, are illustrated in Table 3 (*20*). In a brief report, daily oral intake (12-61 days) of <1 μg/kg body weight 2,3,7,8-tetra-CDD was stated as being lethal to young male rhesus monkeys (*21*), whereas McConnell et al. (*22*) reported an $LD_{50}$ in female rhesus monkeys as <70 μg/kg body weight.

A preliminary report on 2,3,7,8-tetra-CDF concerning the toxicity of PCDFs (*23*), and a report on PCDFs (*24*) have been published.

The toxic syndrome produced by PCDDs and PCDFs may be divided into seven categories:

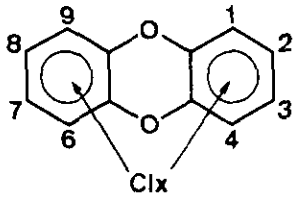
***Chloracne.*** 2,3,7,8-Tetra-CDD and the tri-PCDF and tetra-PCDF were found to be active skin irritants and to induce acneform lesions in the skin of rabbit ears (*25*).

Classical chloracne is a hallmark of PCDD exposure in humans and an analogous hyperkeratosis and the modulation of sebaceous structures to keratin cysts have been observed in monkeys, rabbits, and hairless mice.

Chloracne or acneform dermatitis is a common occupational dermatitis characterized by comedones, keratin cysts, pustules, papules, and abscesses. In 1957, Kimmig and Schulz (*26*) found that 2,3,7,8-tetra-CDD was the agent responsible for causing occupational chloracne in employees of chlorophenol-producing factories. Further, in 1971 PCDDs were implicated as causing chloracne in male workers in a plant producing 2,4-D and 2,4,5-T (*27, 28*). Chloracne may appear weeks or months after the initial exposure to PCDDs and PCDFs.

***Hepatoxicity.*** The degree of hepatic involvement appears to be dose-dependent, and the severity of the changes produced varies between species (*29*). Hepatic necrosis produced by 2,3,7,8-tetra-CDD is probably a contributing cause of death in rats and rabbits, while hepatic necrosis and liver insufficiency are less extensive in mice and are minimal in comparison in guinea pigs and monkeys (*22, 30-37*). Hepatic porphyrin accumulation has been observed in mice, rats, and chickens.

***Hypoplasia of the Lymphoid Tissues.*** Particularly involved are the cortical cells of the thymus and this hypoplasia has been observed in mice, rats, guinea-pigs, and monkeys. The most significant findings in both mice and

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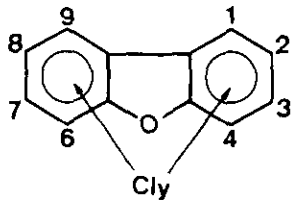


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guinea-pigs treated with sublethal doses of 2,3,7,8-tetra-CDD were in the lymphoid system, resulting in suppression of cell-mediated immunity, particularly in young animals (*36, 38*). Low levels of 2,3,7,8-tetra-CDD that did not produce overt clinical or pathological changes still reduced host defences: 1 μg/kg bw given orally once weekly for 4 weeks to mice before infection with Salmonella increased mortality and decreased the time from infection to death (*39*). The increased mortality may be caused by the endotoxin content of these gram negative bacteria, since 2,3,7,8-tetra-CDD markedly increases the susceptibility of mice to endotoxin (lipopolysaccharide of *Escherichia coli*) (*40*). Treatment of female mice and rats with 2,3,7,8-tetra-CDD during the latter half of gestation and in the postnatal period resulted in a severe depletion of lymphocytes in the thymic cortex of the offspring (*41*). Cellular immunity was impaired.

Hematological changes in mice, rats, and guinea pigs treated with 2,3,7,8-tetra-CDD include lymphopenia and thrombocytopenia (*42, 43*); manifest also is an increased susceptibility to infection concomitant with the suppression of cell-mediated immunity. For 9 months, female rhesus monkeys received a diet containing 500 ppt 2,3,7,8-tetra-CDD; within 6 months, the monkeys became anemic and after 9 months pancytopenic

**Table 1. Possible number of isomers for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.**

| Chlorine atoms | PCDD isomers[a] | PCDF isomers[b] |
|---|---|---|
| 1 | 2 | 4 |
| 2 | 10 | 16 |
| 3 | 14 | 28 |
| 4 | 22 | 38 |
| 5 | 14 | 28 |
| 6 | 10 | 16 |
| 7 | 2 | 4 |
| 8 | 1 | 1 |
| Total | 75 | 135 |

[a] Chlorinated dibenzo-dioxins: empirical formula $C_{12}H_{7-0}Cl_{1-8}O_2$; molecular weight ranges, 218-460.
[b] Chlorinated dibenzofurans: empirical formula, $C_{12}H_{7-0}Cl_{1-8}O$; molecular weight ranges, 202-444.

(*44*). The marked thrombocytopenia was associated with widespread hemorrhage. Death occurred in five of the eight monkeys between months 7 and 12 of the experiment at total exposure levels of 2,3,7,8-tetra-CDD of 2-3 μg/kg body weight. At autopsy, in addition to extensive hemorrhage, there was a distinct hypocellularity of the bone marrow and lymph nodes. Hypertrophy, hyperplasia, and metaplasia of the epithelium in the bronchial tree, bile ducts, pancreatic ducts, salivary-gland ducts, and palpebral conjunctivae were observed. Squamous metaplasia and keratinization of the sebaceous glands and hair follicles were present in the skin. Death was attributed to complications from the severe pancytopenia (*44*).

***General Debilitation and Wasting.*** Animals that receive a toxic or lethal dose of PCDDs or PCDFs exhibit a chronic and progressive weight loss with parallel mobilization of peripheral fat, increased serum triglyceride levels, and development of a fatty liver. Death due to PCDDs or PCDFs intoxication is delayed, as exemplified by an elapsed time period of 6 to 8 weeks following administration of a lethal dose and eventual death (*18, 22, 44*).

***Embryotoxicity and Teratogenicity of 2,3,7,8-tetra-CDD.*** In repeated or single doses of 2,3,7,8-tetra-CDD to mice, as little as 1-10 μg/kg cause increased frequencies of cleft palate and kidney abnormalities (*45-48*) (see Table 4). In rats, embryo-lethal effects occur under experimental conditions (*49, 50*), and kidney anomalies (*45*), intestinal hemorrhages, and general edema can be produced in the fetuses (*51*). Few follow-up studies of the effects of prenatal exposure on postnatal functions have been published. In mice, fetal kidney abnormalities caused by 2,3,7,8-tetra-CDD may progress into a hydronephrosis during the postnatal period (*52*).

***Chick Edema Disease.*** Hydropericardium, ascites, subcutaneous edema, liver necrosis, and death were described in 1957 following the accidental administration of toxic fats in the feed of broiler chickens (ascites have been observed also in mice) (*1*). The toxic material was later identi-

Table 2. Estimated single oral LD50-30 values of certain polychlorinated dibenzo-*p*-dioxin isomers.[a]

| Chlorine isomer | Guinea pigs | | Mice | |
|---|---|---|---|---|
| | μg/kg | μmole/kg | μg/kg | μmole/kg |
| 2,8 | >300,000 | >1,180 | — | — |
| 2,3,7 | 29,444 | 120.41 | >3,000 | >10 |
| 2,3,7,8 | 2 | 0.006 | 283.7 | 0.88 |
| 1,2,3,7,8 | 3.1 | 0.009 | 337.5 | 0.94 |
| 1,2,4,7,8 | 1,125 | 3.15 | >5,000 | >14 |
| 1,2,3,4,7,8 | 72.5 | 0.185 | 825 | 2.11 |
| 1,2,3,6,7,8 | 70-100[b] | 0.178-0.255 | 1,250 | 3.19 |
| 1,2,3,7,8,9 | 60-100[b] | 0.153-0.255 | >1,440 | >3.67 |
| 1,2,3,4,6,7,8 | >600 | >1.400 | — | — |
| 1-$NO_2$-3,7,8 | >30,000 | >90 | — | — |
| 1-$NH_2$-3,7,8 | >30,000 | >99 | — | — |
| 1-$NO_2$-2,3,7,8 | 47.5 | 0.129 | >2,000 | >5.4 |
| 1-$NH_2$-2,3,7,8 | 194.2 | 0.576 | >4,800 | >14.2 |

[a] Data from McConnell et al. (*18*). The $LD_{50-30}$ was calculated by the Spearman-Karber method (*19*).
[b] Estimated range due to variability in replicates.

fied in commercial oleic and stearic acids produced from inedible tallow recovered from animal hides; trichlorophenols and pentachlorophenols had been used in the curing of the hides. The edema causative was termed toxic fat and more specifically chick edema factor and was characterized by x-ray crystallography as 1,2,3,7,8,9-hexa-CDD (*53*). 2,3,7,8-Tetra-CDD, hexa-CDD, and octa-CDD have been also identified in several commercial fatty acids (*54*). Daily doses of 10 or 100 μg hexa-CDD/kg bw, or of 1 or 10 μg 2,3,7,8-tetra-CDD/kg bw, produced a positive response in the chick edema bioassay; 0.5% octa-CDD in the diet had no effect (*35*). Similar edematous effects were observed in rats, pigs, dogs, and monkeys, but not in guinea pigs (*55*). Decreased serum albumin may be associated with the edema; edema has been also shown to occur in chicks dosed orally with 2,3,7,8-tetra-CDF (*56*).

Table 3. Summary of the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin.[a]

| | Mice | Guinea pigs | Monkeys (female) |
|---|---|---|---|
| Thymus involution | +++ | +++ | +++ |
| Spleen reduction (white pulp) | + | + | + |
| Bone marrow hypoplasia | ± | ++ | + |
| Liver megalocytosis/generation | +++ | − | − |
| Bile duct hyperplasia | ± | ± | +++ |
| Testicular degeneration | ++ | +++ | NA |
| Renal pelvis hyperplasia | − | ++ | + |
| Urinary bladder hyperplasia | − | ++ | − |
| Adrenal cortical atrophy (*zona glomerulosa*) | − | ++ | − |
| Hemorrhage | | | |
| Intestinal | + | + | − |
| Adrenal | − | ++ | − |
| Ascites | ++ | − | + |
| Cutaneous lesions | − | − | +++ |

[a] Data from Moore (*20*).

***Other Effects.*** In one or more species of laboratory animals, bone marrow hypoplasia, testicular degeneration, renal pelvis and urinary bladder hyperplasia, and hemorrhage in the intestines and adrenals have been observed.

***Enzyme Induction.*** 2,3,7,8-Tetra-CDD and other halogenated dibenzo-*p*-dioxins and dibenzofurans stimulate a number of enzyme activities, most notably in the liver (*1*). 2,3,7,8-Tetra-CDD is a potent inducer of hepatic and renal microsomal drug metabolizing enzymes (*57-69*). Intoxication with 2,3,7,8-tetra-CDD results in a marked increase in the cellular smooth endoplasmic reticulum content of hepatic and renal cells (*60,69*). 2,3,7,8-Tetra-CDD can simultaneously activate and suppress certain microsome-associated foreign-compound and steroid-hormone-metabolizing enzyme systems (*63*) as well as increase the activity of both renal and hepatic glutathione-S transferase (*70*).

2,3,7,8-Tetra-CDD is the most active of the PCDDs in inducing hepatic δ-aminolevulinic acid (ALA) synthetase and aryl hydrocarbon hydroxylase (AHH) in chick embryo liver preparations (*71, 72*).

The PCDDs that induce ALA synthetase in chick embryo have two common properties: (1) halogen atoms occupy at least three of the four ring positions (2,3,7,8), and (2) at least one free, nonhalogenated carbon atom is unoccupied (*71*). The available toxicological data (*35*) indicate that those PCDDs that are lethal at low doses, teratogenic, or produce acne also induce ALA synthe-

Table 4. Evaluation of reported embryotoxic and teratogenic effects induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxins in rats and mice[a]

| Species | Strain | Embryotoxic/ teratogenic effect | Dose, μg/kg | | Period of dosing, days | Route | Reference |
|---|---|---|---|---|---|---|---|
| | | | Lowest Tested[b] | $ED_{50}$[c] | | | |
| Rat | | Intestinal hemorrhage | 0.125 | 0.5? | | Oral | (*50*) |
| | CD | Kidney abnormality | 0.5 | >1 | 6–15 | S.C. | (*45*) |
| Mouse | CD-1 | Cleft palate | 1? | | | | |
| | | | 3 | >3 | 6–15 | S.C. | (*45*) |
| | | Kidney abnormality | 1 | 1–3 | 6–15 | S.C. | (*45*) |
| | DBA/2J | Cleft palate | 3 | >3 | 6–15 | S.C. | (*45*) |
| | | Kidney abnormality | 3 | >3 | 6–15 | S.C. | (*45*) |
| | C57BL/6J | Cleft palate | 3 | >3 | 6–15 | S.C. | (*45*) |
| | | Kidney abnormality | 3 | <3 | 6–15 | S.C. | (*45*) |
| | NMRI | Cleft palate | 3 | 6.5 | 6–15 | Oral | (*46*) |
| | | | 9 | <9 | 9–13 | Oral | (*46*) |
| | | | 15 | 40 | 13 | Oral | (*47*) |
| | | | 5 | 15 | 11 | Oral | (*47*) |

[a] Data from Neubert et al. (*47*).
[b] The lowest dose with which an embryotoxic or teratogenic effect detectable at birth has been produced is indicated. Since sometimes only one dose level was tested, this does not necessarily represent the lowest dose from which an effect could result.
[c] $ED_{50}$-dose required to produce an embryotoxic effect in 50% of animals.

tase; those PCDDs that are not toxic generally do not induce ALA synthetase. The structure-activity relation of PCDDs to induce AHH in chick embryos was identical to that in inducing ALA synthetase (*71*).

Mixed-function oxidase enzyme systems of mouse strains "non-responsive" to other aromatic hydrocarbons were induced by single doses of 2,3,7,8-tetra-CDD, as evidenced by increases in hepatic monooxygenase activities and in concentrations of cytochrome P-448 (*73–76*). Genetic resistance to induction of AHH by 3-methylcholanthrene in DBA/2J mice was overcome by treatment with 2,3,7,8-tetra-CDD. This result conflicts with the hypothesis that induction of AHH activity is a consequence of the formation of cytochrome P-448 (*77*). A component that has a high binding affinity for 2,3,7,8-tetra-CDD was found in mouse liver cytosol (*78*).

2,3,7,8-Tetra-CDD induces AHH even in 'poorly responsive' strains of mice (*79*), not only in liver but also in lung, kidney, and colon. The DBA/2N strain, which responds only weakly to the sarcomatogenic action of 3-methylcholanthrene, becomes highly susceptible after treatment with 2,3,7,8-tetra-CDD (*74, 80*).

2,3,7,8-Tetra-CDD is approximately 30,000 times more potent than 3-methylcholanthrene in inducing AHH activity in rat liver (*81*).

McConnell et al. (*18*) reported that the comparative toxicity of 13 PCDDs in mice and guinea-pigs (see Table 2) supports the idea that the relative potency (or rank order) of a congener to produce one toxic response is a good indicator of its relative potency (or rank order) to produce other toxic manifestations.

***Hepatic Cytosol Binding Protein.*** A macromolecular binding species has been characterized in the hepatic cytosol fraction of mouse and rat liver which has the *in vitro* binding properties predicted for the receptor for the induction of AHH activity based on the *in vivo* biology (*78*): namely, (1) $^3$H-2,3,7,8-tetra-CDD binds to this cytosol protein reversibly with a high affinity ($K_d$ = 0.27 *nM*) comparable to the $ED_{50}$ for hepatic AHH induction ($ED_{50}$ in mice = 1 nmole/kg); (2) the binding affinity of halogenated dibenzo-*p*-dioxins and dibenzofurans for this protein *in vitro* corresponds to their potency to induce hepatic AHH activity in the chicken embryo; and (3) other compounds, such as the polycyclic aromatic hydrocarbons, which induce AHH activity and cytochrome $P_1$-450 also compete for this cytosolic binding protein, but compounds which induce other types of microsomal monooxygenase activities (e.g., phenobarbital) and steroids fail to bind. Thus, this cytosolic binding protein may be the receptor for the induction of AHH activity.

***Structure-Activity Relations.*** The pathologic effects produced by the toxic PCDD and PCDF isomers are similar to those of 2,3,7,8-tetra-CDD for a given species, differing only in the intensity of the toxic effect produced by a given isomer. The toxic PCDDs have chlorine atoms in at least three of the four lateral ring positions (2,3,7, and 8) with at least one unsubstituted ring position (the octa-CDD is comparatively inactive). To the extent that the toxicity has been determined, a

similar structure-toxicity relation has been observed for the PCDFs.

The structure-activity relation established for PCDDs and PCDFs for the induction of hepatic aryl hydrocarbon hydroxylase (AHH) activity and for binding to the hepatic cytosol binding species has been extended to other classes of chlorinated aromatic compounds. 3,4,3′4′-Tetrachloroazoxybenzene (TCAOB) and 3,4,3′,4′-tetrachloroazobenzene (TCAB) are potent acnegens formed as trace contaminants in the synthesis of 3,4-dichloroaniline or herbicides based on this compound (*82*). At high doses in animals, TCAB is reported to produce thymic involution and liver damage similar to 2,3,7,8-tetra-CDD (*83*). Both TCAOB and TCAB are potent inducers of hepatic AHH activity and bind to the hepatic cytosol binding protein with a high affinity. Congeners such as 3,5,3′5′-tetrachloroazoxybenzene and 3,5,3′,5′-tetrachloroazobenzene fail to induce AHH activity, fail to bind to the hepatic cytosol species, and fail to produce chloracne.

Of 16 halogenated biphenyl compounds tested, only 3,4,3′4′-tetrachloro-, 3,4,5,3′4′5′-hexachloro- and 3,4,5,3′4′5′-hexabromobiphenyls induced hepatic AHH activity and bound to the hepatic cytosol binding species (*84*). The 3,4,3′4′-tetrachlorobiphenyl has been reported to produce chloracne. McKinney et al. (*56*) found that of five hexachlorobiphenyls tested in chickens, 3,4,5,3′4′5′-hexachlorobiphenyl was by far the most toxic, and the only one that induced significant chick edema and involution of the thymus.

***Pharmacokinetics.*** In the rat, following acute or chronic administration, 2,3,7,8-tetra-CDD is accumulated primarily in the liver and to a lesser extent in the fat, and is largely eliminated unmetabolized in the feces with a whole body half-life of about 3 weeks. Some pharmacokinetic experiments suggest the formation of a polar metabolite appearing in the urine, but direct attempts to demonstrate metabolism with hepatic microsomes *in vitro* have been negative (*1*).

***Mutagenicity.*** Only four dioxin isomers have been evaluated for mutagenicity: the 2,7-di-, 2,3,7,8-tetra, and octa-CDDs as well as the unsubstituted dibenzo-*p*-dioxin (*85*).

2,3,7,8-Tetra-CDD increased the reversion frequency to streptomycin independence in *Escherichia coli* Sd-4. In *Salmonella typhimurium*, frameshift mutations in strain TA1532, but not base substitutions in strain TA1530, were induced by toxic concentrations of 2,3,7,8-tetra-CDD (*86*). In plate assays, the response was positive with *S. typhimurium* TA1532, doubtful with TA1531 and TA1534, and negative with G46 and TA1530 (*87*). McCann (*88*) tested 2,3,7,8-tetra-CDD in *S. typhimurium*, both with and without metabolic activation, using a spot-test and the standard plate test with strains TA1532, TA1535, TA1537, and TA1538; all these tests were negative.

Octa-CDD was nonmutagenic in *S. typhimurium* strains G46, TA1530, and TA1531, and doubtful results were obtained with strains TA1532 and TA1534 (*87*). Metabolic activation systems were not included in any of these microbiological assays.

Inhibition of mitosis and chromosomal abnormalities (dicentric bridges and chromatin fusion with formation of multinuclei or a single large nucleus) were observed in endosperm cells of the African blood lily (*Haemanthus Katherinae* Baker) treated with 2,3,7,8-tetra-CDD in the presence or absence of 2,4,5-T (*89*).

No chromosomal aberrations were observed in bone marrow cells of male rats treated with 2,7-di-CDD, 2,3,7,8-tetra-CDD, or dibenzo-*p*-dioxin by oral intubation, with 2,3,7,8-tetra-CDD by intraperitoneal injection or orally (*90*). However, when Osborne Mendel rats of both sexes were treated twice weekly for 13 weeks with 2,3,7,8-tetra-CDD, a significant but weak increase in the number of chromosome aberrations in bone marrow cells was reported (*91*).

2,3,7,8-Tetra-CDD did not induce dominant lethal mutations in Wistar rats after oral administration to males for 7 days (*51*).

***Carcinogenicity.*** Two reports indicate that chronic administration of low levels of 2,3,7,8-tetra-CDD to rats is associated with an increased incidence of neoplasia (*1, 92, 93*).

Groups of 10 male Sprague-Dawley rats were fed a diet containing 2,3,7,8-tetra-CDD for 78 weeks in the following amounts (figures in parentheses are approximate weekly doses): 0, 1 ppt (0.0003 μg/kg body weight) 5 ppt (0.001 μg/kg), 50 ppt (0.01 μg/kg), 500 ppt (0.1 μg/kg), 1 ppb (0.4 μg/kg), 5 ppb (2.0 μg/kg), 50 ppb (24 μg/kg), 500 ppb (240 μg/kg), and 1000 ppb (500 μg/kg). The three highest dose levels (50, 500, and 1000 ppb) were toxic and killed all animals by the fourth week. Of the six remaining test groups, the overall incidence of neoplasms was 23/60 (38%); none occurred in the 1 ppt group. In the 5 ppt group, 5/10 animals had 6 neoplasms [earduct carcinoma, lymphocytic leukemia, adenocarcinoma, malignant histocytoma (with metastases), angiosarcoma, Leydig-cell adenoma]; the following groups also showed neoplasms: 50 ppt, three observed in 3/10; 500 ppt, four 4/10; 1 ppb, five observed in 4/10; 5 ppb, ten observed in 7/10. Neoplasms were not observed in the controls (*92*).

Groups of 100 Sprague-Dawley rats (50 males and 50 females) received for two years diets containing 0, 22, 210, and 22,000 ppt, equivalent to 0.0, 0.001, 0.01, and 0.1 μg 2,3,7,8-tetra-CDD/kg/day. Continuous ingestion of 0.001 μg/kg/day did not cause any chemically related changes in tumor incidence or toxicity; feeding with 0.01 μg/kg/day induced an increased incidence ($p < 0.05$) of hepatocellular hyperplastic nodules (female: 18/50 versus 8/86 controls), of focal alveolar hyperplasia in the lungs, and of urinary excretion of porphyrins (female). Dietary intake of 0.1 μg/kg/day caused an increased incidence ($p < 0.05$) of hepatocellular carcinomas (female: 11/49 versus 1/86) and squamous-cell carcinomas of the lung (female: 7/49 versus 0/86), of the hard palate/nasal turbinates (male: 4/50 versus 0/85; female: 4/49 versus 0/86), and of the tongue (male: 3/50 versus 0/85). Further increased were adenoma of the adrenal cortex (male) and hepatocellular hyperplastic nodules (female). At this dose, certain age-related lesions were reduced (males: acinar adenoma of the pancreas; females: granulosal cell neoplasm of the ovary, benign and malignant tumors of the mammary gland, pituitary adenoma, and benign tumors of the uterus). Also, chronic administration of 2,3,7,8-tetra-CDD caused multiple toxicologic effects, including increased mortality, decreased body weight gain, slight depression of certain hematologic parameters, increased urinary excretion of porphyrins and δ-aminolevulinic acid, increased serum levels of alkaline phosphatase, glutamyl transferase and serum glutamic pyruvic transaminase, and morphologic changes primarily of the hepatic, lymphoid, respiratory, and vascular tissues of the body (*93*).

These two reports show that chronic administration of 2,3,7,8-tetra CDD causes an increased incidence of neoplasms, but not whether 2,3,7,8-tetra-CDD acts as an initiator or promoter. This consideration is particularly important because unequivocal evidence is lacking that 2,3,7,8-tetra-CDD is a mutagen or is metabolized, and no evidence is available that 2,3,7,8-tetra-CDD and/or metabolite(s) bind covalently to macromolecules.

As summarized in Table 5, at least 24 long-term carcinogenicity studies on mice and rats are in progress (*94*).

## Humans

***Toxic Effects in Humans.*** Toxicity due to 2,3,7,8-tetra-CDD has been reported after occupational exposure during the industrial synthesis of 2,4,5-trichlorophenol (TCP) and 2,4,5-T, after exposure in factories and in the surrounding environment due to accidents occurring during the synthesis of TCP, and after exposure to herbicides and other materials containing 2,3,7,8-tetra-CDD. Exposed subjects have been found to develop a wide variety of lesions and symptoms (Table 6). For instance, a typical exposure victim experiences a number and a variety of clinical signs and symptoms: early exposure symptoms may include a burning sensation of the eyes, nose, and throat followed by headache, dizziness, nausea, and vomiting. Some days later, severe itching, redness, swelling of the face, more marked over the eyelids, nose and lips, may develop. Within the initial weeks after exposure, inflamed nodules as well as pustules appear on the face, forearms, shoulders, neck, and trunk, leading then to comedones and cysts. After a month or two, acneform eruptions emerge and the skin becomes hyperpigmented. At about the same time, aching muscles, mainly in the thighs and chest area, become manifest and aggravated on exertion. Insomnia, extreme irritability, and loss of libido also occur during this stage.

Other than the consistently found clinical feature of acne, other findings in humans may include: neuromuscular symptoms (weakness and pain with nerve conduction abnormalities), porphyria cutanea tarda, hepatic dysfunctions, hyperlipidemia, cutaneous hyperpigmentation and hirsutism, chronic eye irritation, emotional disorders, and neuropsychiatric syndromes.

Chloracne, one of the most constant and prominent features of 2,3,7,8-tetra-CDD exposure, has been described as a refractory acne characterized by inclusion cysts, comedones and pustules, with eventual scarring of the skin, more frequently originating on the face and sometimes spreading to other parts of the body. Many patients also have blepharoconjunctivitis and irritation of other mucous membranes. Sometimes the chloracne is preceded by erythematous and edematous skin lesions. The latent period between exposure and the appearance of clear signs of chloracne ranges from a few weeks to several months (*106*).

An important and unique episode revolves around three scientists who were self-exposed to 2,3,7,8-tetra-CDD: one heated trichlorophenol in an alkaline solution, a second heated potassium trichlorophenate, and a third worked in the same laboratory as the second and used a diluted solution of the synthesized dioxin (*107*). The first two scientists developed chloracne eight weeks after exposure, whereas the third showed no evidence of the characteristic acneform lesions. Delayed symptoms, probably due to 2,3,7,8-tetra-CDD, developed approximately two years later, and the second two scientists showed personality changes

Table 5. Ongoing long-term carcinogenicity testing of chlorinated dibenzo-p-dioxins.[a,b]

| Compound | Number of studies | Route | Species |
|---|---|---|---|
| Dibenzo-*p*-dioxin | 4 | Oral (diet) | Mouse, rat |
| | | Skin | Mouse |
| | | | Mouse (with DMBA)[c] |
| 2,7-Di-CDD | 4 | Oral (diet) | Mouse, rat |
| | | Skin | Mouse |
| | | | Mouse (with DMBA)[c] |
| 2,3,7-Tri-CDD | 3 | Oral (gavage) | Mouse, rat |
| | | Skin | Mouse |
| 2,3,7,8-Tetra-CDD[d] | 5 | Oral (diet) | Rat |
| | | Oral (gavage) | Mouse |
| | | Oral (gavage) | Mouse, rat |
| | | Skin | Mouse |
| 1,2,3,6,7,8-Hexa-CDD[d] | 3 | Oral (gavage) | Mouse, rat |
| | | Skin | Mouse |
| 1,2,3,7,8,9-Hexa-CDD[d] | 2 | Oral (gavage) | Mouse |
| | | Skin | Mouse (with 1,2,3,6,7,8-hexa-CDD) |
| 1,2,3,4,6,7,8,9-Octa-CDD | 3 | Oral (diet) | Mouse, rat |
| | | Skin | Mouse |

[a] Data taken from Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity, No. 7 (*94*). Details of these studies as well as the authors/institutes are available in this source reference document.

[b] As of October 1979, carcinogenesis bioassay testing within the National Toxicology Program included unsubstituted dibenzo-*p*-dioxin (UDD; CAS 262-12-4; NCI Tech. Rept. No. 122), considered not carcinogenic to Osborne-Mendel rats and B6C3F1 mice when administered in feed at dose levels of 5 and 10 ppb, and 2,7-dichlorodibenzo-*p*-dioxin (DCDD; CAS 22857-26-0; NCI Tech. Rept. No. 123), considered not carcinogenic to Osborne-Mendel rats and female B6C3F1 mice but suggestive of a carcinogenic effect in male B6C3F1 male mice when fed concentrations of 5 and 10 ppb. Studies are in progress on 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HCDD, CAS 57653-85-7) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD: CAS 1746-01-6) by gavage to Osborne-Mendel rats and B6C3F1 mice and by skin painting on Swiss mice. Testing on 2,3,7,8-tetrachlorodibenzofuran (TCDF; CAS 51207-31-9) is to begin in fiscal year 1980. For chemical disposition studies octachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and the stereochemically related 2,4,3',4'-tetrachloroazobenzene are also proposed.

[c] DMBA = dimethylbenzanthracene.

[d] The National Toxicology Program has completed and reported the results for the long-term carcinogenesis bioassay studies on 2,3,7,8-tetrachlorodibenzo-p-dioxin and 1,2,3,6,7,8-/1,2,3,7,8,9-hexachlorodibenzo-p-dioxins, with the following results.

2,3,7,8-tetrachlorodibenzo-p-dioxin—gavage, carcinogenic for Osborne-Mendel rats (increased incidences of follicular-cell thyroid tumors in males and of liver tumors in females) and carcinogenic for B6C3F1 mice (liver tumors in both sexes and thyroid tumors in females); dermal, carcinogenic for female Swiss-Webster mice (fibrosarcoma in the integumentary system) and although not shown carcinogenic for male Swiss-Webster mice, an increase in the same tumor type was observed. Mixture of hexachlorodibenzo-p-dioxins—gavage, carcinogenic for female Osborne-Mendel rats (hepatocellular carcinomas or neoplastic nodules), carcinogenic for male and female B6C3F1 mice (hepatocellular carcinomas and adenomas), and not demonstrated as carcinogenic for male Osborne-Mendel rats; dermal—not considered carcinogenic for male and female Swiss-Webster mice.

(mainly loss of energy and drive); impairment of vision, taste, and muscular coordination; sleep disturbances; gastrointestinal symptoms; and hirsutism. The first of the three experienced none of these adverse effects. All three exhibited hypercholesterolemia (>300 mg/100 ml).

## Human Exposure to PCDDs and PCDFs

Major sources of human exposure to PCDDs and PCDFs include: exposure in the workplace; exposure in factories and in the surrounding environment from industrial accidents; exposure to contaminated materials, wastes, or food in the general environment; and exposure in Vietnam and other intensive herbicide spraying operations. (Prior and subsequent to use as a defoliant, 2,4,5-T was used in weed-killing and forest-thinning operations in the United States of America and elsewhere).

Occupational exposure may occur in manufacturing plants producing chlorinated phenols (tri-, tetra-, and pentachlorophenols), or phenoxy acid herbicides (2,4-D, 2,4,5-T), or PCBs; in factories utilizing these chemicals for the production of other substances (hexachlorophene from 2,4,5-trichlorophenol); in factories manufacturing or repairing transformers and capacitors or having heat exchange or heat hydraulic systems containing PCBs; and in the use processes of these chemicals under various occupational conditions such as spraying of herbicides, using chlorinated phenols for a variety of applications (especially wood preservative), sawing, or otherwise processing treated wood, and using hexachlorophene in sanitary occupations.

Table 6. Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxins in humans.

| Effects | References |
|---|---|
| Dermatological | |
| Chloracne | (*25, 28, 95-108*) |
| Porphyria cutanea tarda | (*27, 104, 105, 109*) |
| Hyperpigmentation and hirsutism | (*27, 28, 107*) |
| Internal | |
| Liver damage[a] | (*27, 95, 98, 101, 102, 104-106, 110*) |
| Elevated serum hepatic enzyme levels | (*27, 28, 96, 104-106, 110*) |
| Disorders of fat metabolism | (*28, 104, 107*) |
| Disorders of carbohydrate metabolism | (*28, 101, 102, 104, 105*) |
| Cardiovascular disorders | (*101, 102, 104, 105*) |
| Urinary tract disorders | (*97, 101, 102*) |
| Respiratory disorders | (*95, 101, 102*) |
| Pancreatic disorders | (*101, 102*) |
| Neurological | |
| Polyneuropathies (peripheral neuritis) | (*101, 102, 104, 105*) |
| Lower extremity weakness | (*28, 95, 99, 101, 102, 104, 105, 108*) |
| Sensory impairments (sight, hearing, smell, taste) | (*28, 101, 102, 107, 110*) |
| Psychiatric | |
| Neurasthenic or depressive syndromes | (*28, 95, 99, 101, 102, 104, 105, 107*) |

[a] Mild fibrosis, fatty changes, hemofuscin deposition and parenchymal-cell degeneration were observed in a few cases.

The burning of materials impregnated with commercial 2,3,4,6-tetrachlorophenates yielded 150-1000 μg of mixed PCDDs/g chlorophenate. Although only found as a minor constituent, 2,3,7,8-tetra-CDD has been quantified at levels exceeding 10 μg/g chlorophenate (*111*).

Pyrolysis of a technical grade PCB mixture yielded many PCDF isomers; the total yield could be as high as 3-25%. One of the main constituents is 2,3,7,8-tetra-CDF, the most toxic PCDF-isomer. Consequently, uncontrolled burning of PCBs can be an important environmental source of the hazardous PCDFs, and operations such as welding or soldering electrical equipment containing PCBs, or using casting waxes in foundries, may possibly entail a significant exposure (*112*). A PCB used in a heat exchange system for two years contained approximately 1.25 ppm of 2,3,7,8-tetra-CDF and a total of 15 ppm of PCDFs (*113*).

Apart from accidents such as the one which occurred in Seveso in 1976, general environmental exposure may originate from herbicide spraying and waste disposal.

An outbreak of PCDDs poisoning in humans, horses, and other animals occurred in Missouri in 1971 (*97, 114*) following the spraying of contaminated oil for dust control in horse arenas.

Another possible source of exposure to PCDDs and PCDFs pollution are waste oils, and possibly other waste, when burned both in industrial and municipal incinerators. Under simulated environmental conditions, the combustion of a standard 2,4,5-T formulation led to formation of small amounts of PCDDs and PCDFs (*115*). Buser and Bosshardt (*11*) quantified the total amount of PCDDs and PCDFs in fly ash from an industrial incinerator heating facility as 0.2 and 0.1 ppm, and in the fly ash from an industrial heating facility in Switzerland as 0.6 and 0.3 ppm. More than 30 individual PCDDs can be identified in the fly ash, but the known highly toxic PCDD isomers are only minor constituents (*12*). The number of PCDF isomers was larger; but, in this case, the known highly toxic isomers are major constituents (*13*).

An additional potential source of human exposure has been revealed: beef fat taken from cattle grazed on 2,4,5-T-treated rangeland contained 2,3,7,8-tetra-CDD; of the 11/14 positive samples, the four with the highest levels had 12, 20, 24, and 70 ppt (*10*). In another study, 3 of 24 samples of beef fat contained 3-4 ppt 2,3,7,8-tetra-CDD (*116*). Moreover, in a preliminary report of an ongoing enlarged study of women living in areas where 2,4,5-T is used on rangeland, 4/18 breast milk samples each contained approximately 1 ppt (*10*).

Workers in wood processing industries are exposed to wood dust containing preservatives as well as accompanying impurities and degradation products. Wood dust from a saw-mill in which a 2,3,4,6-tetrachlorophenol formulation was used as a fungicide was found to contain 1-10 ppm PCDFs and <0.5 ppm PCDDs (*15*).

In 1968, more than 1200 persons in South-west Japan were intoxicated by consuming a commercial rice oil contaminated with 1000 ppm PCBs. Nagayama et al. (*117*) analyzed the rice oil (Yusho oil) and found 5 ppm PCDFs, the major constituents of which were tetra- and penta-CDFs. Buser et al. (*113*) recently showed that 2,3,7,8-tetra-CDF was the main PCDF in the Yusho oil (0.45 ppm). The high level of PCDFs was caused by leakage from heat exchangers containing PCBs contaminated with PCDFs.

Workers manufacturing 2,4,5-trichlorophenol (TCP) or 2,4,5-T during normal production operations, and/or following explosions taking place in these plants, may have been exposed to a variety of polychlorinated chemicals whose type and

quantity depends on the particular chemical processes in use and on the phase of the reaction in which the accident took place. Apparently, for instance, PCDFs are produced in the earlier stages when mainly tetrachlorobenzene is present and PCDDs are produced nearer the end of the reaction when primarily trichlorophenol is extant. The concentration of chlorophenols and chlorobenzenes, for example, was probably higher in the 1963 episode in The Netherlands, where the accident took place at the beginning of the reaction, then in the 1968 episode in the United Kingdom, or the 1976 accident in Italy, where the explosion took place at the end.

A thorough description of the reported industrial accidents and other cases of intoxications observed in exposed workers is given in the IARC Monograph (*1*). In this present report, only those episodes specifically discussed at length by the NIEHS/IARC *ad hoc* Working Group will be considered. (A sequential perspective of known accidents is given in Table 7).

In 1949, the first reported cases of industrial poisoning due to the formation of 2,3,7,8-tetra-CDD in uncontrolled exothermic reactions occurred during the manufacture of 2,4,5-trichlorophenol at a 2,4,5-T-producing factory in Nitro, West Virginia; 288 persons were affected (*99, 118*). In November 1953, an accident occurred in Ludwigshafen, Federal Republic of Germany, during the manufacture of TCP (*101,102,119,120*); 53 workers were affected by chloracne.

Five cases of chloracne were reported following an industrial accident in an Italian TCP-producing factory (*121*). In 1963, an accident occurred at the 2,4,5-T-producing factory in The Netherlands; approximately 50 persons were affected by chloracne (*122, 123*). In 1966, Dugois et al. (*98*) observed 21 cases of chloracne after an accident in a French factory producing TCP in the Grenoble region. In 1968, an accident occurred at the TCP-producing factory at Bolsover, Derbyshire, United Kingdom (*103, 106*). Within the next 7 months, 79 workers developed chloracne. In 1971, 2,3,7,8-tetra-CDD contaminated waste oil caused an outbreak of poisoning in humans, horses, and other animals (*97, 124*). In July 1976, an accident at the TCP-producing factory in Meda, Italy (*96*) resulted in the contamination of a large, densely populated area, including the towns of Seveso, Meda, Cesano Maderno, and Desio.

Key information concerning the effects of human exposure to herbicides in Vietnam, especially to the so-called "Agent Orange" (a 50:50 mixture of the *n*-butyl esters of 2,4-D and 2,4,5-T, containing up to 30 mg/kg or more 2,3,7,8-tetra-CDD), may be found in the literature (*1, 110, 125-127*).*

## Exposure Episodes Considered in Detail by the NIEHS/IARC *ad hoc* Working Group

The text below is a condensed version of the discussions which took place during the two-day NIEHS/IARC *ad hoc* Working Group Meeting.

### Phenoxy Acids Exposure

Cohort studies on herbicide sprayers have been conducted or are being planned in Scandinavian countries. The more advanced of these concerns Swedish railroad workers with exposure to a variety of herbicides. These people exhibited a significantly increased tumor incidence (apparently dose-dependent) and tumor mortality (*129*). The excess of tumors was found particularly among workers with exposure to amitrole (aminotriazole), whereas those exposed to phenoxy acids (2,4-D and 2,4,5-T) showed only a slightly increased excess of cancers. The study was small-sized, comprising 2978 person-years at observation in the total cohort and with 18 deaths *versus* 20.54 expected. The original conclusion from this study was that amitrole exposure may have caused an excess of tumors, whereas there was probably no pertinent increase in tumor incidence associated with exposure to phenoxy acids. The study has been recently reanalysed using a case-control approach, and through stratification on amitrole when considering the effect from phenoxy acids, and *vice versa*. The results show a possible and previously masked tumor-inducing effect also from phenoxy acids.

Another retrospective cohort mortality study, conducted in Finland on workers of five companies involved in spraying 2,4-D and 2,4,5-T on brushwood, did not show any increase in mortality. During 1955-1971, in the younger group of workers (under 45 years of age), however, four cancer deaths were observed versus less than two expected (no statistically significant difference). A prospective follow-up study for the period 1972-1976 revealed fewer cancer-related deaths than expected in all age groups. Clinical and anamnes-

* More than 2,000,000 gallons of Agent Orange remaining from military defoliant operations were destroyed by incineration on board ship in the Pacific Ocean, 120 miles from Johnston Island. Temperatures not lower than 1250°C were used. The steel containers were melted (*128*).

tic investigations were also performed showing a picture of acute complaints during and following the spraying operations: headache, transient dizziness, fatigue, abdominal complaints, skin and mucous irritations, and a few cases of persistent papulae.

Interestingly, some samples of 2,4,5-T used in Finland for spraying operations contained from 0.04 to 0.07 ppm 2,3,7,8-tetra-CDD. This may imply that the 2,4,5-T produced before 1965 contained lower levels of 2,3,7,8-tetra-CDD impurities than has been observed subsequently from 2,4,5-T used elsewhere. However, the analytical methods used in the determination of 2,3,7,8-tetra-CDD in the Finnish samples were antiquated in view of the rapid advancements made in analytical methodology and thus a confirmatory analysis of the samples using modern techniques was made by Rappe et al. (*130*). Five samples of 2,4,5-T ester dating from 1962 to 1967 were analysed for PCDDs and PCDFs; levels of 0.1-0.95 ppm 2,3,7,8-tetra-CDD and 0.1-0.15 ppm tetra-CDF were found.

A second feasibility study was designed and conducted in Finland to determine whether one could obtain anamnestic, clinical, hematological, and immunological information on Finnish railway and forestry workers who had been exposed for several years to herbicides containing 2,3,7,8-tetra-CDD. The exposed group consisted of 30 men who were control-matched to persons of the same age coming from the same district with similar job backgrounds and living conditions. Of the tests made, including liver and immunofunction studies, no differences were observed between the exposed and control groups. Although the population study is too small to permit any general conclusions as to the potential adverse health effects from long-term exposure to phenoxy acids, the investigation shows that follow-up is possible on the health status of persons exposed for several years to 2,3,7,8-tetra-CDD-containing phenoxy acids, and then to compare the exposed group results to carefully matched controls. The study is in progress.

## Hexachlorophene Exposure

An excess of malformations, some severe, has been reported among children whose mothers were employed as nurses in a hospital. The mothers were exposed to hexachlorophene soap during early pregnancy, and the hypothesis of a causal relation between such an exposure and the occurrence of the malformations has been advanced (*131*); five severe and six slight malformations were observed in 65 children in the exposed group, whereas only one slight malformation was observed in the 68 children of the unexposed group. This report has been followed by another study (to be published) of a similar group of children of exposed mothers in comparison to children of unexposed mothers. Again a high frequency of malformations among the offspring of exposed mothers was reported (*132*). From these studies, however, it is not clear if exposure to other hazardous chemicals could be excluded. A retrospective-prospective study is being initiated among long-term workers of an United States' factory which used Seveso-produced 2,4,5-trichlorophenol in the manufacture of hexachlorophene. However, in the case of hexachlorophene, polychlorinated xanthenes have been also identified as contaminants at a higher level than 2,3,7,8-tetra-CDD (*133*).

## Phenoxyacids and Chlorophenols Exposure in Forestry and in Wood Industry Workers

At the Regional Hospital in Umea (Northern Sweden), Hardell (*134*) observed that several patients suffering from mesenchymal tumors reported occupational or other exposures to phenoxyacids. More specifically, 87 mesenchymal tumors were diagnosed during the years 1970 through 1976. Of these cases, 55 were men (more than expected) and 43 of these 55 men were known by profession: 19 were either forestry workers, farmers and forestry workers, or workers in sawmills and papermills where exposure to chlorophenols is common. Based on the official statistics of Sweden, one can calculate approximatively the expected fraction of tumors within these trades, resulting in an expectancy of approximately 11 cases versus the 19 observed.

The Mount Sinai School of Medicine in New York is currently planning a field survey on long-term health effects on wood-preservative workers in Arkansas and Oklahoma in the United States of America.

## Exposure to 2,3,7,8-Tetra-CDD during Production of 2,4,5-T or Polychlorinated Phenols and Following Industrial Accidents

The following cases, as well as others not considered at the meeting, have been described in IARC Monographs Volume 15 (*1*), and only some

of the previously available information will be given here. The ongoing epidemiological studies on these cohorts are summarized in Table 7.

As each individual cohort has a relatively small number of person-years, a study has been proposed in which all the cohorts would be pooled and the mortality compared to that expected from the national statistics.

The first reported cases of industrial poisoning due to the formation of 2,3,7,8-tetra-CDD in uncontrolled exothermic reactions occurring during the manufacture of TCP were seen in 1949 at a 2,4,5-T-producing factory in Nitro, West Virginia, USA; 228 persons were affected. These individuals were studied by Suskind and Ashe (*135*) and followed up for a period of four years. Symptoms included chloracne, nausea, vomiting, headaches, severe muscular aches and pain, fatigue, emotional instability, and intolerance to cold. Laboratory findings showed raised total lipids and raised prothrombin time. Among those affected were not only workmen, but also laboratory personnel, medical personnel, and even the Safety Director who visited the areas of exposure. Several wives who had never visited the plant also developed acne, usually at the same time as their employee husbands.

It is important to note that information on the toxicity as well as on the stability (long persistence and slow degradability) of 2,3,7,8-tetra-CDD was not available at the time the first reports of acute toxicity were observed, and no measures were taken to decontaminate the factories and control the residue levels of 2,3,7,8-tetra-CDD. In fact, the presence of 2,3,7,8-tetra-CDD or other PCDDs and PCDFs was not even suspected. Workers, therefore, were probably exposed for a considerable time to 2,3,7,8-tetra-CDD following the first observations of acute toxic effects.

In November 1953, an accident occurred at a TCP factory in Ludwigshafen, Federal Republic of Germany; 75 workers were exposed to the reaction products during the accident and the subsequent cleaning work; most were affected by chloracne, 42 severely: 21 of the 42 suffered consequent damage to internal organs or disturbances of the nervous system. The most relevant features were polyneuritis, sensorial impairments, and liver damage. The son of one of the workers developed chloracne following contact with his father's work clothes. An additional case of poisoning occurred 5 years later in a worker who was involved in repair work (welding one of the auto-claves) at the contaminated site; subsequently this worker died with necrotic pancreatitis.

All the 75 workers could be traced in a cohort study 25 years later; mortality was compared both with the regional and national mortality and with a control group of workers from the same factory. In the exposed group, 17 deaths were observed (11 to 25 expected depending on the control population); six from cancer (four or less expected), five from cardiovascular diseases (as expected), two from suicides (0.2-0.6 expected), one from liver cirrhosis, one from a urogenital tract disease, and two from traffic accidents.

Of the six cancer deaths, three were from stomach cancer and occurred in the age group of 60-69, which is significantly more than expected. This effect was more pronounced when a minimum observation period of 10 years was considered, and more so for stomach cancer after a 20-year observation period. (Use of a long observation period permits a better estimation of the relation between exposure and disease when long "latency" periods are involved, as for cancers).

A similar accident occurred at the 2,4,5-T-producing factory in The Netherlands in 1963; 106 men are likely to have been exposed to 2,3,7,8-tetra-CDD (and possibly PCDFs) as they were in the building where the accident occurred during the subsequent period, March-July 1963. These workers may be divided into three groups: (A) factory employees (exposed during all phases of the cleaning and reconstruction activities), 44 men; (B) clean-up crew (nonfactory personnel, exposed during the months May-July), 18 men; and (C) plumbing, painting, and insulating personnel (nonfactory workers, exposed during the months May-July), 44 men.

During the explosion in Amsterdam, two operators were present in the building. With the exception of these men, who were probably exposed primarily by the inhalation route, all other workers used safety masks and were probably exposed by dermal contact. Chloracne was the most common and prominent lesion observed following 2,3,7,8-tetra-CDD exposure (26/44 examined in group A, 10/16 in group B, and 8/16 in the group C). Liver function tests (thymol turbidity and serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase) did not indicate liver damage. A few men complained of fatigue. The latent period between exposure and the appearance of chloracne, characterized by comedones, pustules, and cysts on the face and sometimes on other parts of the body, was approximately 4-6 weeks. Sometimes, erythematous and edematous skin lesions, possibly due to phenolic compounds, were noted one day after exposure. No skin lesions (chloracne) were reported

in family members of the employees. Of group A, 3/44 workers died, one of pancreas carcinoma in 1964, one of myocardial infarction at the age of 69, and one of a traffic accident. Of the 17/18 traced individuals in the B group, four have died (three from sudden death, probably of myocardial infarction, at ages, 41, 53, and 65, and one of ill-defined pulmonary or cardiac cause at age 44). Only 32/44 of the C group were traced, and one of them died of sudden death, probably myocardial infarction, at age 50.

Jirásek et al. (*104, 105, 109*) studied 55 subjects from a cohort of 78 workers from a factory in Czechoslovakia producing 2,4,5-T and pentachlorophenol, who were affected by chloracne. The workers were probably exposed during the years 1965-1968 to unknown levels of 2,3,7,8-tetra-CDD. A significant symptom was the disturbance in porphyrins metabolism with signs of porphyria cutanea tarda. Many workers also suffered severe hepatic and neurological damage and had raised blood levels of cholesterol and total lipids. In the first years of follow-up, five deaths were observed, two from bronchogenic carcinoma at age 47 and 59 (only 0.12 lung cancer deaths were expected from national mortality statistics), one from atypical atherosclerosis at age 57, and one from the acute occupational intoxication. One further death, probably from liver cirrhosis, has recently been observed and 50 workers are still being followed. Many workers continue to show lipemia and hypercholesterolemia and have developed prediabetic changes and hypertension. The workers lost to follow-up are mostly foreigners who returned to their country of origin.

In July 1976, an accident in a TCP-producing factory in Meda, Italy resulted in the contamination of the towns of Seveso, Meda, Cesano Maderno, Desio, Nova Milanese, and possibly others of a large, densely populated area called the Brianza di Seveso with a total population of about 220,000 inhabitants. A retrospective health survey conducted on the workers of the ICMESA factory revealed that two workers among those directly involved in the production of 2,4,5-trichlorophenol suffered from skin lesions previous to July 1976. These facts may be indicative of a previous 2,3,7,8-tetra-CDD exposure.

A large and intensive follow-up program is in progress in the Seveso area; data were presented about the first dermatological, neurological, clinical, and laboratory findings, as well as about fetal damage. Dermatological examinations, including the screening of 32,000 children, resulted in the finding of hundreds of children with skin lesions, 135 of which were eventually identified as chloracne; 59 cases were from the more contaminated area (Zone A). However, precise description of diagnostic criteria and accurate anamnestic information, including, among others, data on places of residence, were not included. This information is needed before evaluating the meaning of the chloracne incidence data by territorial areas and efforts should be made obtain it. Chloracne cases were not systematically searched for in adults. Neurological examinations showed both signs of subclinical neurological damage and cases of clinically detectable polyneuropathy in adults (34/338 persons from zone A and 8/185 from the surrounding areas).

In about 30% of 1654 adults from both A zone and nearby zones, hepatomegaly was reported to be present on clinical investigation. No information, however, is given on the criteria by which the hepatomegaly was evaluated. The same percentage of abnormalities was observed in one or more liver tests (mainly glutamyl transferase and serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase). Extensive monitoring of various hematological parameters from 25,000 persons is now in progress and the data are expected to become available during 1978. Results of this investigation should allow a better understanding as to whether or not these alterations are caused by exposure to 2,3,7,8-tetra-CDD and PCDDs.

So far, immunological investigations, cytogenetic research, and embryomorphology analyses on cases of therapeutical abortions have not given abnormal results. However, the study of the frequency of congenital malformations and spontaneous abortions seems to be very difficult to accomplish. The importance of a systematic study on this subject has been stressed by the Working Group, because the questions on the teratogenicity of 2,3,7,8-tetra-CDD in humans cannot be solved by any other study.

A preliminary mortality study has been possible in 2 of the 11 towns of the Seveso area. The 1975-1977 mortality rates from liver cirrhosis and leukemia in these two limited zones of the Seveso area were compared to that of a nearby area (province of Varese). Within an overall mortality which did not appear different, an increase of deaths from liver cirrhosis and from leukemia was noted. The validity and significance of these observations should be carefully evaluated.

## Recommendations

A chemical analytical program should be encouraged to identify and standardize analytical

**Table 7. Epidemiological studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; human exposure episodes caused by accidental or occupational exposure occurring in factories producing 2,4,5-trichlorophenol and/or 2,4,5-trichlorophenoxyacetic acid.**

| Town and country | Year of exposure | Number of exposed persons | Clinical follow-up and cohort studies | Observations to date | Proposed actions | Comments and recommendations of the NIEHS/IARC Working Group |
|---|---|---|---|---|---|---|
| Accidental exposures | | | | | | |
| Nitro, West Virginia, (U.S.A.) | 1949 | 228 | Only 36 individuals were followed, for a period of 4 years | No significant long-term observations are yet available (some followed-up individuals had symptoms of severe intercostal neuritis | Mortality cohort study being planned | Efforts should be made to trace the individuals who were exposed, possibly through the employment compensation files. |
| Ludwigshafen/Rhein, Federal Republic of Germany | 1953 | 75 | A mortality cohort study has conducted 24 yr after the accident; all exposed workers were traced for a total of 1525 person-years of observation | 6 Cancer deaths were observed: 3 of stomach cancer in the age group 60–69 (significantly higher than expected), 2 of oat-cell carcinoma of the lung, and 1 of colonic adenocarcinoma | Follow-up to be continued | The results of the continuing follow-up study should periodically be made available. |
| Amsterdam, The Netherlands | 1963 | 106 | Preliminary observations on mortality are available; 93 exposed workers have been traced up to 1977 | Of 8 deaths, 5 or 6 were from cardiovascular diseases, 5 probably from myocardial infarctions; the myocardial infarction death rate seems higher in the most heavily exposed workers affected by chloracne. One worker, who did not develop chloracne, died from pancreatic carcinoma 14 months after the accident; however, he complained of abdominal pain prior to the accident | Full report planned for 1978 | Efforts should be made to trace all the workers who were exposed. |
| Bolsover, Derbyshire, United Kingdom | 1968 | 90 | Of the workers belonging to the original cohort, 50% are still employed and routinely undergo clinical and laboratory investigations | One worker is known to have died from coronary thrombosis | Epidemiological cohort study to be planned when the results of the present clinical follow-up investigation become available | Efforts should be made to trace all the workers who were exposed. |

| | | | | | | |
|---|---|---|---|---|---|---|
| Meda, Brianza di Seveso, Italy | 1976 | ?[a] | A clinical follow-up of the inhabitants from the more contaminated areas is being carried out. At the time of the accident, living in Zone A was a population of 730 people, with 200 of those being between 0–14 years of age. Of these 730 persons, 623 (125 between 0–14 years of age) were followed-up. Zone B contained a population of 4732 inhabitants with 1293 between the ages 0–14 years. An accurate count of those followed in Zone B is not available, but 943 youngsters between 0–14 years of age are included in the follow-up | Initial clinical observations showed a high incidence of 2,3,7,8-tetra-CDD-dependent skin lesions among children living in the more contaminated area. Also, a number of persons residing in the zone surrounding the more contaminated areas had skin lesions, but with poorly defined, clinical features; these lesions may be the result of 2,3,7,8-tetra-CDD exposure. Moreover, both clinical and laboratory findings suggest an increased incidence of hepatic sufference among adults living in the highly contaminated areas. A preliminary mortality study suggests that although the overall rates from 2 Seveso towns are not different from a nearby area, an increase of deaths from liver cirrhosis and leukemia was noted | Among other ongoing investigations, a long-term morbidity study has been initiated | Possible teratogenic and other adverse reproductive and developmental effects from 2,3,7,8-tetra-CDD exposure should be investigated. A mortality register and population files, suitable for adequate descriptions of morbidity data, should be established. Further, the cancer registry that already covers a nearby province, should be extended to include the Seveso area. |
| Occupational exposure Czechoslovakia | 1965–1969 | 80 | A follow-up has been made regularly on 55 workers of the original cohort. The workers lost to follow-up are mainly foreigners who left the country | Five patients died during the first 5 year-follow-up period: 2 died from bronchogenic carcinoma and 1 from a rapidly developing atypical atherosclerosis. One further death, probably from liver cirrhosis, has recently occurred. Many of the workers are now suffering from hypertension and show signs of hyperlipidemia and hypercholesterolemia as well as prediabetic symptoms and signs | Follow-up to be continued. A 10-year report will be prepared during 1978 | The results of the continuing follow-up study should periodically be made available. |

[a] No reliable information was available to the Working Group about the actual number of exposed persons; the entire area has a population of approximately 200,000 people.

methods used, to catalogue products contaminated with PCDDs and PCDFs, and to establish a register of known PCDD and PCDF standard samples.

The Working Group unanimously recommended that a system be developed for an international exchange of information and research coordination on the health effects of chlorinated dibenzo-*p*-dioxins/chlorinated dibenzofurans. Further, and in particular, the following three areas were indicated as deserving special attention:

1. Development of sensitive and reliable measurements of body burden; improved, more sensitive, and less expensive analytical technology should be generated. Standardized sampling (air, water, soil, tissues and body fluids, environmental), preparation, clean-up, and analysis should be promoted;

2. Development of common protocols for clinical examinations (including reproductive experience) and, in particular, design of a protocol for basic core information;

3. Development of an international registry of exposed persons to serve as a basis for long-term follow-up: this was considered especially important because the relatively small size of populations involved in individual exposure episodes are an obstacle to risk assessment and thus pooling of data is almost a necessity.

The Working Group recommended that IARC explore the technical and financial feasibility, possibly in cooperation with other national and/or international organizations, of setting-up such a permanent mechanism which could include as a minimum starting program: (a) a periodical, perhaps biennial, meeting to up-date the progress of ongoing studies, and to identify needed developments; (b) an exchange of relevant information accruing during the interval between meetings; and (c) a register of chloracne cases, containing simple, essential information in a standardized form. This PCDDs/PCDFs exposure-related sign may help to better define an exposed cohort population suitable for long-term follow-up.

This report represents the views and opinions of the U.S. NIEHS/IARC (National Institute of Environmental Health Sciences/International Agency for Research on Cancer *ad hoc* Working Group which met January 10-11, 1978 in Lyon France to review the history of human exposure to these chemicals, to collate current information, and to plan and forecast needed new directions. The meeting was funded jointly by NIEHS and IARC.

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