INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

ENVIRONMENTAL HEALTH CRITERIA 88

POLYCHLORINATED DIBENSO- *PARA*-DIOXINS AND DIBENZOFURANS

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    Every effort has been made to present information in the criteria
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errors that may have occurred to the Manager of the International
Programme on Chemical Safety, World Health Organization, Geneva,
Switzerland, in order that they may be included in corrigenda, which
will appear in subsequent volumes.

                          *   *   *

    A detailed data profile and a legal file can be obtained from the
International Register of Potentially Toxic Chemicals, Palais des
Nations, 1211 Geneva 10, Switzerland (Telephone No. 7988400 -
7985850).

ENVIRONMENTAL HEALTH CRITERIA FOR POLYCHLORINATED DIBENZO-PARA-
DIOXINS AND DIBENZOFURANS

A WHO Task Group on Environmental Health Criteria for
Polychlorinated Dibenzo-para-dioxins and Dibenzofurans met at the
Monitoring and Assessment Research Centre, London, United Kingdom,
from 9 to 13 February, 1987. Dr M. Berlin opened the meeting and
welcomed the members on behalf of the host Institute and on behalf of
the United Kingdom Department of Health and Social Security, who
sponsored the meeting. Dr G.C. Becking addressed the meeting on behalf
of the three cooperating organizations of the IPCS (UNEP, ILO, and
WHO). The Task Group reviewed and revised the draft criteria document
and made an evaluation of the risks for human health and for the
environment from exposure to polychlorinated dibenzo-p-dioxins and
dibenzofurans.

The drafts of this document were prepared by Dr U.G. Ahlborg, Dr
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Environmental Medicine, Stockholm, Sweden, and by Professor C. Rappe
of the University of Umea, Umea, Sweden.

The efforts of all who helped in the preparation and finalization
of the document are gratefully acknowledged.

<center>* * *</center>

ABBREVIATIONS

AHH   aryl hydrocarbon hydroxylase
ALA   aminolevulinic acid
BGG   bovine gammaglobulin
BHA   butylated hydroxyanisole
BP   benzo(a)-pyrene
CMI   cell-mediated immunity
DEN   diethylnitrosamine
diCDD   dichlorinated dibenzo-p-dioxin
diCDF   dichlorinated dibenzofuran

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DMBA      dimethylbenzathraline
ECOD      7-ethoxycoumarin-o-deethylase
EGF       epidermal growth factor
EH        epoxide hydratase
EI        electron impact
EROD      7-ethoxyresurofin-o-deethylase
ETG       epidermal transglutaminase
fg        femtogram (10^{-15}g)
GC        gas chromatography
heptaCDD  heptachlorinated dibenzo-p-dioxin
heptaCDF  heptachlorinated dibenzofuran
hexaCDD   hexachlorinated dibenzo-p-dioxin
hexaCDF   hexachlorinated dibenzofuran
HMI       humoral-mediated immunity
HPLC      high pressure liquid chromatography
IARC      International Agency for Research on Cancer
ip        intraperitoneal
IR        infrared
LOEL      lowest-observed-effect level
MCPA      4-chloro-o-tolyloxyacetic acid
MFO       mixed-function oxidase
MS        mass spectrometry
MSW       municipal solid waste
ng        nanogram (10^{-9}g)
NMR       nuclear magnetic resonance
NOEL      no-observed-effect level
octaCDD   octachlorinated dibenzo-p-dioxin
octaCDF   octachlorinated dibenzofuran
PAH       polyaromatic hydrocarbons
PCB       polychlorinated biphenyl
PCDD      polychlorinated dibenzo-p-dioxin
PCDF      polychlorinated dibenzofuran
PCDPE     polychlorinated diphenylether
PCPY      polychlorinated pyrene
PCQ       polychlorinated quaterphenyl
pentaCDD  pentachlorinated dibenzo-p-dioxin
pentaCDF  pentachlorinated dibenzofuran
pg        picogram (10^{-12}g)
SC        subcutaneous
SCE       sister chromatid exchange

SD        standard deviation
SEM       standard error of the mean
SIM       selected ion monitoring
TCDD      2,3,7,8-tetrachlorinated dibenzo-p-dioxin
TCDF      2,3,7,8-tetrachlorinated dibenzofuran
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TCP        trichlorophenol
tetraCDD   tetrachlorinated dibenzofuran
tetraCDF   tetrachlorinated dibenzofuran
TPA        12-o-tetradecanoylphorbol-13-acetate
triCDD     trichlorinated dibenzo-p-dioxin
triCDF     trichlorinated dibenzofuran
t3         triiodothyronine
t4         thyroxine
UDPGT      UDP-glucuronosyltransferase
UV         ultraviolet
2,4-D      2,4-dichlorophenoxyacetic acid
2,4,5-T    2,4,5-trichlorophenoxyacetic acid
3-MC       3-methylcholanthrene
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1. SUMMARY AND RECOMMENDATIONS

1.1 Summary

1.1.1 Sources

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated
dibenzofurans (PCDFs) are two series of tricyclic aromatic compounds
with similar chemical and physical properties; they are ubiquitous in
the environment. They do not occur naturally, nor are they
intentionally produced. There are 75 positional isomers of PCDDs and
135 isomers of PCDFs.

The most important sources of contamination with PCDDs and PCDFs
include:
-    contaminated commercial chemical products, such as
     chlorinated phenols and their derivatives, and PCBs;
-    incineration of municipal, hazardous, and hospital
     wastes, and of sewage sludges;
-    automobile operation;
-    fossil fuel combustion;
-    overheating and emissions from fires involving PCBs;
-    disposal of industrial wastes resulting from
     processes such as the production of chlorophenols and
     their derivatives, chlorophenol wood treatment, use
     of PCB fluids in electrical equipment, and wastes
     from pulp and paper processing.

1.1.2 Ambient levels and routes of exposure

The limited data available indicate that ambient levels of these
compounds are very low in air, soil, and sediment, i.e. fg/m^3 in

air, ng/kg in soil and sediment. Levels of PCDDs and PCDFs up to 50 ng/kg have been found in aquatic organisms in the general environment. Data on contamination of drinking water and commercial food are very limited.

Exposure to these compounds in the general population probably occurs mainly through the food-chain.

Some workers engaged in the production, use, and destruction of materials containing PCDDs and PCDFs and their precursors may receive high exposure. For these persons, inhalation and dermal contact are the primary exposure routes of concern.

1.1.3 Toxicokinetics, biotransformation, and biological monitoring

The bioavailability of PCDDs and PCDFs depends on the matrix they are in and the route of exposure. Data on bioavailability through inhalation are not available for any species.

The quantity absorbed by humans after any route of exposure is not known.

Studies on rodents given single or repeated oral doses of 2,3,7,8-TCDD have shown that about half of the administered dose is absorbed from the gastrointestinal tract. The reported half-lives for elimination were between 12 and 94 days for rodents. The half-life of 2,3,7,8-TCDD in adipose tissue of the rhesus monkey is about 1 year.

Animal data on the toxicokinetics of PCDDs other than 2,3,7,8-TCDD are limited. The half-life for 2,3,7,8-TCDD has been reported to be in the range of 2 and 8 days for rats, mice, and monkeys and more than 20 days for guinea-pigs. Studies on rats have shown that 2,3,4,7,8-pentaCDF is more highly retained than is 2,3,7,8-TCDD.

Data on the retention of PCDDs and PCDFs in tissues of various species, exposed to synthetic mixtures or to environmental samples containing PCDDs and PCDFs, show a high variability in retention time between congeners with or without chlorine substitution in the 2,3,7, and 8 positions.

Limited human data indicate half-lives for some 2,3,7,8-substituted PCDDs and PCDFs in the range of 2-6 years.

The PCDDs and PCDFs are predominately stored in fat, but they are also excreted in milk and pass through the placenta. They also appear

in the blood and vital organs at lower concentrations.

The tissue distribution in humans is not clear at present, although it has been suggested that the ratio between fatty tissue and liver is higher in humans than in rodents.

In human fat, background levels of TCDD up to 20 ng/kg have been found in the general population, with no known specific exposure, but higher levels have been reported in some cases without evidence of disease. None of these populations were randomly sampled. The more highly chlorinated PCDDs and PCDFs, particularly octaCDD, are also present in these samples. Average tissue levels of TCCD tend to increase with age.

1.1.4 Health effects

1.1.4.1 Animals

The toxic and biological effects resulting from exposure to 2,3,7,8-TCDD are dependent on a number of factors, which include the species, strain, age, and sex of the animals used. The toxic responses observed in several animal species include body weight loss, hepatotoxicity, porphyria, dermal toxicity, gastric lesions, thymus

atrophy and immunotoxicity, teratogenicity, reproductive effects, and carcinogenicity. TCDD induces a wide spectrum of biological effects including enzyme induction and vitamin A depletion. Not all of these effects are observed in any single animal species. The most characteristic toxic effects observed in all laboratory animals are body weight loss, thymus atrophy, and immunotoxicity. Chloracne and related dermal lesions are the most frequently noted signs of 2,3,7,8-TCDD toxicosis in humans; dermal lesions are also observed in rhesus monkeys, hairless mice, and rabbits. In contrast, most rodents do not develop chloracne and related dermal toxic lesions after exposure to 2,3,7,8-TCDD. Many of the toxic lesions are noted primarily in epithelial tissues.

Reproductive effects have been reported in rhesus monkeys and rats. The lowest-observed-effect levels have been reported to be approximately 1-2 ng/kg body weight per day. In two cancer studies in rats, hepatocellular carcinomas were produced at approximate dose levels of 0.1 µg/kg body weight per day and 0.01 µg/kg body weight per day. Doses of 0.001 µg/kg body weight resulted in foci or areas of hepatocellular alteration. The incidence of certain hormone-dependent tumours was lower than in the control animals.

TCDD does not appear to have mutagenic properties, and is therefore not likely to be genotoxic. Thus, it is assumed to be carcinogenic through an indirect mechanism.

Several other PCDDs and PCDFs cause signs and symptoms similar to those of 2,3,7,8-TCDD, but there is a wide variation with regard to potency. There are 12 isomers that display higher toxicity, i.e., the tetra-, penta-, hexa-, and heptaCDDs and CDFs with four chlorine atoms in the symmetrical lateral positions 2,3,7, and 8. A mixture of two hexachlorodibenzo-p-dioxins (1,2,3,7,8,9- and 1,2,3,6,7,8-hexaCDD) has been demonstrated to possess carcinogenic properties in long-term animal studies, but at higher doses than those used in the study of TCDD. Dibenzo-p-dioxin and 2,7-diCDD failed to demonstrate carcinogenic properties. The relative toxic and biological potencies of PCDDs and PCDFs have been estimated using short-term studies in rats and mammalian cell cultures.

There are marked species differences in the susceptibility of animals to the biological and toxic effects elicited by 2,3,7,8-substituted PCDDs and PCDFs. For example, the oral LD50 values range from 0.6 µg/kg body weight in guinea-pigs, to 5051 µg/kg body weight in Golden Syrian hamsters for 2,3,7,8-TCDD. The tremendous variation in species and strain sensitivity to 2,3,7,8-TCDD and related compounds cannot be explained by the observed toxicokinetic differences. The toxicity and toxicokinetics of TCDD in monkeys most closely resemble the effects observed in humans. There is evidence in

inbred mice that the cellular levels of the Ah receptor correlate, in part, with susceptibility to the biological and toxic effects of these compounds. The receptor has also been identified in other species including man. However, interspecies comparison of cellular Ah receptor levels do not explain fully the differences in sensitivity.

1.1.4.2 Humans

For occupational and accidental exposures to PCDDs and PCDFs, in spite of many clinical and follow-up studies, no clear-cut persistent systemic effects have been delineated except for chloracne. Other effects have been noted, but, apart from chloracne and perhaps minor functional disorders, none has been persistent.

In some epidemiological studies of people exposed to a mixture of dioxins, furans, and other chemicals, an increased incidence of cancer at different sites has been claimed, but a number of factors limits confidence in the findings.

In the Seveso accident, the only clear-cut adverse health effect recorded has been chloracne. Chloracne (193 cases) occurred in 1976 and 1977, and 20 of those individuals still had active chloracne in 1984. Many studies have been performed to find possible links between exposure to Agent Orange and health effects in civilians or military personnel in Viet Nam. However, the information available to date does not allow definite conclusions to be drawn with regard to effects on human reproduction or any other significant health effects.

In the Missouri incident, children who showed acute illness when the contamination occurred in 1971 are now reportedly in good health. Furthermore, epidemiological studies in Missouri on populations exposed to lower concentrations of dioxins over longer periods of time have so far not revealed any significant health effects. Although no clinical symptoms were observed, there were indications of an effect on the cell-mediated immune system.

The only documented intoxications with PCDFs in humans are the two instances of contamination of rice oil with PCDFs, PCBs, and PCQs, i.e., Yusho in Japan, 1968, and Yu-cheng in Taiwan, 1979. In total, several thousand people were acutely intoxicated. From the data it appears most likely that the causative agent was the PCDFs. The general symptomatology was similar to that seen in intoxications with TCDD, with the differences reflecting the intensity of exposure and the ages and sex of those exposed.

The average daily intake of 2,3,7,8-substituted PCDFs by Yusho patients was estimated to be 0.1-0.2 µg/kg body weight for a period of several months, while the lowest dose causing disease was estimated to be 0.05-0.1 µg/kg body weight per day over a period of 30 days.

1.1.5 Conclusion

PCDDs and PCDFs occur throughout the environment and we all probably carry a body burden of them. They have sometimes produced complex toxic effects following occupational and accidental exposure.

Based on the Yusho disease and experiments in sensitive species of monkeys, and making assumptions about the relative potencies of PCDDs and PCDFs, man and certain monkeys may have comparable sensitivity to these compounds. However, the uncertainties related to the real dose received by humans and the difficulties of assessing toxic effects other than chloracne in humans prevents a firm conclusion as to the relative resistance of humans to the toxic effects of these compounds. Exposure should be reduced to levels as low as reasonably practicable.

1.2   Recommendations

1.   Analytical interlaboratory validation and "round-robin" studies
using standardized quality assurance and quality control procedures
are needed to improve analytical methodology.

Sampling strategy and analytical procedures and data
interpretation should be optimized and standardized before undertaking
surveys.

2.   Further information is required about the origins and
environmental distributon and fate of PCDDs and PCDFs.

Further monitoring data, including time trends and determinations
of isomer patterns, are required for environmental levels of PCDDs and
PCDFs, especially for food, ambient air, and sediments.

3.   Data should be obtained about the effects of PCDDs and PCDFs on
environmental biota.

4.   More information is required on the bioavailability of PCDDs and
PCDFs from different matrices in the environment and from the diet.
Exposure from these sources should be correlated with agricultural and
industrial practices.

5.   Simpler and less expensive chemical and biological methods
suitable for screening for the presence of PCDDs and PCDFs should be
developed and validated.

6.   Studies to determine the mechanisms of toxicity of PCDDs and
PCDFs are needed to support an evaluation of the differences in
effects between species and to support an extrapolation to man.

7.   Further investigation of immunotoxicity is important, including
cytotoxic T-lymphocyte function. Studies of the effects of perinatal
exposure and of the duration of actions on the immune system are
important.

8.   Long-term toxicity studies should be carried out, including
multigeneration reproductive studies in different species with three
of the most widespread PCDDs and PCDFs, namely 2,3,4,7,8-pentaCDF,
1,2,3,7,8-pentaCDD, and octaCDD.

9.   Because humans are exposed to complex mixtures of PCDDs and
PCDFs, test systems, including human cell culture systems, should be

developed further and validated for evaluating the toxic potency of
these compounds and other mixtures. These systems can be used to study
mechanisms of action, structure activity relationships, and
interactive effects.

10.   Investigations to examine the body burden and to correlate it
with clinical effects and laboratory findings are indicated. Follow-up
studies of previously exposed groups are important.

2.   IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1   Identity

The polychlorinated dibenzo-para-dioxins (PCDDs) and
polychlorinated dibenzofurans (PCDFs) are two series of almost planar
tricyclic aromatic compounds with very similar chemical properties.
The general formulae are given in Fig. 1.

PCDDs
Dioxins $x + y = 1 - 8$

PCDFs
Dibenzofurans   WHO 88835
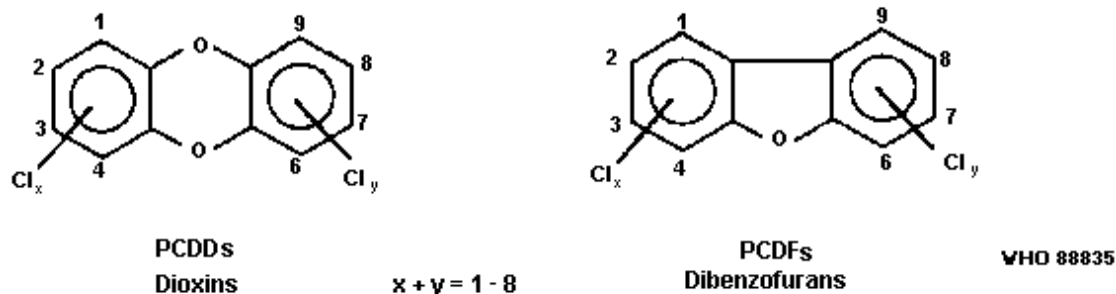
Fig. 1.   Structural formulae of PCDDs and PCDFs.

The number of chlorine atoms can vary between 1 and 8. The term
isomers refers to comparisons between compounds with the same
empirical formulae. The term congeners refers to comparisons between
compounds within the same series but with a different number of
chlorine atoms. The number of positional isomers is quite large; in
all there are 75 PCDDs and 135 PCDFs and the number of isomers for a
certain number of chlorine atoms is given in Table 1.

The nomenclature used in this document is based on the system

used by Chemical Abstracts. The Chemical Abstracts System Registry Numbers (CAS RN) for a few PCDDs and PCDFs that have been cited in the literature are provided in Table 2.

2.2 Physical and Chemical Properties

A large number of the individual PCDDs have been synthesized by various methods and characterized, mainly by gas chromatography-mass spectrometry (GC/MS) (Buser & Rappe, 1980, 1984; Taylor et al., 1985; Rappe et al., 1985a) but also by using nuclear magnetic resonance (NMR) or ultraviolet (UV), infrared (IR), (Pohland & Yang, 1972; Kende et al., 1974), or X-ray analyses (Boer et al., 1973; Slonecker et al., 1983).

Table 1. Number of PCDD and PCDF isomers

Number of chlorine atoms	Number of PCDD isomers	Number of PCDF isomers
1	2	4
2	10	16
3	14	28
4	22	38
5	14	28
6	10	16
7	2	4
8	1	1
	75	135

Table 2. CAS RN for some PCDDs and PCDFs

PCDD congener	CAS RN	PCDF congener	CAS RN
2,3,7,8-TetraCDD	1746-01-6	2,3,7,8-TetraCDF	51207-31-9
1,2,3,7,8-PentaCDD	40321-76-4	1,2,3,7,8-PentaCDF	57117-41-6
1,2,3,6,7,8-HexaCDD	57653-85-7	2,3,4,7,8-PentaCDF	57117-31-4
1,2,3,7,8,9-HexaCDD	19408-74-3	1,2,3,4,7,8-HexaCDF	70648-26-9
1,2,3,4,6,7,8-HeptaCDD	35822-46-9	1,2,3,6,7,8-HexaCDF	57117-44-9
1,2,3,4,7,8,9-HeptaCDD	58200-70-7	1,2,3,7,8,9-HexaCDF	72918-38-8
OctaCDD	3268-87-9	2,3,4,6,7,8-HexaCDF	60851-34-5

Pyrolysis of chlorinated phenols yields small amounts of one or more PCDD isomers. Using this technique all the 22 tetraCDDs have been prepared (Nestrick et al., 1979; Buser & Rappe, 1980) as well as the 14 pentaCDDs (Buser & Rappe, 1984) and 10 hexaCDDs (Lamparski & Nestrick, 1981; Buser & Rappe, 1984).

Taylor et al. (1985) have synthesized, separated, and isolated all the 22 tetraCDD isomers. In Table 3 are listed some other isomers that have been synthesized and isolated.

The most toxic and most extensively studied representative of the chlorinated dioxins (PCDDs) is 2,3,7,8-tetrachlorodibenzo-para-dioxin (2,3,7,8-tetraCDD) (Fig. 2). It is commercially available, as are more than 10 other PCDD congeners.

The empirical formulae, molecular weights, and some physical properties of a few PCDDs are given in Table 4.

Table 3. Synthetic method and melting point for some PCDDs

PCDD Isomer	Synthetic method[a]	Melting point °C	Reference
1-Chloro-	1	80-90	Pohland & Yang, 1972
2-Chloro-	1	88-89	Pohland & Yang, 1972
1,3-Dichloro-	1	113.5-114.5	Kende et al., 1974
2,3-Dichloro-	1	163-164	Pohland & Yang, 1972
2,7-Dichloro-	2	209-210	Pohland & Yang, 1972
2,8-Dichloro-	3	150.5-151	Pohland & Yang, 1972
1,2,4-Trichloro-	4	128-129	Pohland & Yang, 1972
2,3,7-Trichloro-	1	157-158	Kende et al., 1974
2,3,7,8-Tetrachloro-	2	305-306	Pohland & Yang, 1972
2,3,7,8-Tetrachloro-	5	305-307	Kende et al., 1974
1,2,3,4-Tetrachloro-	4	188-190	Pohland & Yang, 1972
1,3,7,8-Tetrachloro-	1	193.5-195	Kende et al., 1974
1,3,6,8-Tetrachloro-	2	219-219.5	Pohland & Yang, 1972
1,2,3,4,7-Pentachloro-	5	195-196	Kende et al., 1974
1,2,3,4,7,8-Hexachloro-	5	275	Pohland & Yang, 1972
1,2,4,6,7,9-Hexachloro-	2	238-240	Pohland & Yang, 1972
Octachloro	2	330	Pohland & Yang, 1972

a Synthetic methods as follows:

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1 = Catechol + chlorobenzene
2 = Pyrolysis of chlorphenols
3 = Cyclization of chlorophenoxyphenol
4 = Catechol + chloronitrobenzene
5 = Chlorination of chlorodibenzodioxin
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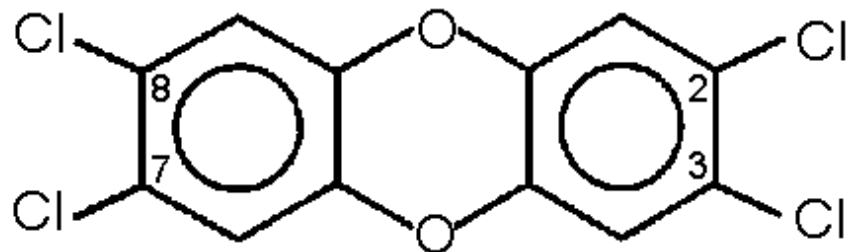

WHO 89037

Fig. 2. Structural formula of TCDD.

Table 4.   Physical properties of some PCDDs

Compound	Molecular formulae	Molecular weight	Absorption maximum (chloroform) (nm)	Reference
2,3,7,8-TCDD	$C_{12}H_4Cl_4O_2$	321.9	310	Pohland & Yang (1972)
1,2,3,7,8-PentaCDD	$C_{12}H_3Cl_5O_2$	356.5	308	Gray et al.

				(1976)
1,2,3,6,7,8-HexaCDD	$C_{12}H_2Cl_6O_2$	390.9	316	Gray et al. (1975)
1,2,3,7,8,9-HexaCDD	$C_{12}H_2Cl_6O_2$	390.9	317	Gray et al. (1975)

Although tetraCDD is lipophilic, it is only slightly soluble in most solvents and very slightly soluble in water (Table 5).

Table 5.   Solubility of 2,3,7,8-tetraCDD in various solvents[a]

Solvent	Solubility at 25 °C	
	g/litre	g/kg
O-Dichlorobenzene	1.8	1.4
Chlorobenzene	0.8	0.72
Perchloroethylene	0.68	0.48
Chloroform	0.55	0.37
Benzene	0.47	0.57
Acetone	0.09	0.11
Dimethylsulfoxide[b]	< 0.1	< 0.1
Methanol	0.01	0.01
Water	2×10^{-7}	2×10^{-7}

[a]   From: Crummett & Stehl (1973).

[b]   DMSO caused detector fouling and a better value could not be obtained.

Table 6.   Water solubility of PCDDs[a]

Compound	Water solubility (g/litre)	
	20.0 °C	40.0 °C
1,3,6,8-TetraCDD	$(3.2\pm0.2) \times 10^{-7}$	$(3.9\pm0.4) \times 10^{-7}$
1,2,3,7-TetraCDD	$(4.3\pm0.1) \times 10^{-7}$	$(12.7\pm0.8) \times 10^{-7}$
1,2,3,4,7-PentaCDD	$(1.2\pm0.1) \times 10^{-7}$	$(4.6\pm0.1) \times 10^{-7}$

```
      1,2,3,4,7,8-HexaCDD        (4.4±0.1) x 10⁻⁹        (19.0±0.1) x 10⁻⁹
  1,2,3,4,6,7,8-HeptaCDD        (2.4±0.3) x 10⁻⁹         (6.3±0.2) x 10⁻⁹
                 OctaCDD        (0.4±0.1) x 10⁻⁹         (2.0±0.2) x 10⁻⁹
```

a From: Friesen et al. (1985).

Marple et al. (1986a) have reanalysed the water solubility of 2,3,7,8-TCDD and found it to be considerably less (12.5-19.2 ng/litre). The log water-octanol partition coefficient (K_{ow}) has been determined as 6.64 by Marple et al. (1986b).

Friesen et al. (1985) have determined the water solubility for some PCDDs other than the 2,3,7,8-TCDD compound and these are given in Table 6.

Similarly Webster et al. (1985) have determined the log octanol-water partition coefficients for a number of PCDDs (Table 7).

2,3,7,8-TetraCDD is considered to be a stable compound, but due to its extreme toxicity its chemistry has not been fully evaluated. However, it undergoes substitution reactions (Baughman, 1974) as well as photochemical dechlorination (Crosby et al., 1971; Crosby & Wong, 1977; Gebefugi et al., 1977). Thermally it is very stable and rapid decomposition of 2,3,7,8-tetraCDD occurs only at temperatures above 750 °C (Stehl et al., 1973). The other PCDDs have been much less studied; however, octaCDD is completely destroyed by treatment with hot alkali (Albro & Corbett, 1977).

The first synthesis of 2,3,7,8-tetraCDD was reported by Sandermann et al. (1957), who used catalytic chlorination of the unchlorinated dioxin. It has also been prepared in good yields by the dimerization of 2,4,5-trichlorophenol salts (Buu-Hoi et al., 1971b; Langer et al., 1973).

In the PCDF series, Mazer et al. (1983) synthesized all the 38 positional tetraCDF isomers. The products were mixtures of isomers, and each of these isomers could be identified. Later Bell & Gari, (1985) isolated and characterized all the 38 tetraCDFs, 28 pentaCDFs, and 16 hexaCDFs.

Table 7. Values for log K_{ow} for some PCDDs from linear and quadratic plots

Compound	log K_{ow} (linear)		log K_{ow} (quadratic)	
	Waters Bondapak	Waters Bondapak (Woodburn data)	Waters Bondapak	Waters Bondapak (Woodburn data)
Dibenzo-p-dioxin	4.26	4.01	4.34	4.17
1-MonoCDD	4.81	4.52	4.91	4.75
2-MonoCDD	5.33	5.00	5.45	5.29
2,7-DiCDD	6.27	5.86	6.39	6.17
1,2,4-TriCDD	7.36	6.86	7.45	7.11
1,2,3,7-TetraCDD	8.15	7.58	8.19	7.72
1,2,3,4-TetraCDD	8.63	8.02	8.64	8.07
1,3,6,8-TetraCDD	8.70	8.08	8.70	8.12
1,2,3,4,7-PentaCDD	9.48	8.80	9.40	8.64
1,2,3,4,7,8-HexaCDD	10.40	9.65	10.22	9.19
1,2,3,4,6,7,8-HeptaCDD	11.38	10.55	11.05	9.69
OctaCDD	12.26	11.35	11.76	10.07

a   From: Webster et al. (1985).

Kuroki et al. (1984) have synthesized 51 congeners of PCDFs by a structure specific method from chlorophenols and chloronitrobenzenes or chlorophenols and chlorodiphenyls iodonium salts. The structures were confirmed by MS and NMR.

Safe & Safe (1984) described the synthesis of 22 PCDF congeners resulting in quantities of 10-320 mg of purified product. They also reported NMR data on the compounds synthesized.

Sarna et al. (1984) and Burkhard & Kuehl, (1986) have documented the octanol/water partition coefficients for some PCDFs (Table 8). The disagreement for OCDF arises because of uncertainties in the K_{ow} values of reference compounds of high K_{ow}. The partitioning of organic chemicals between lipid and water is an important determinant of the bioconcentration potential of a toxicant and has sometimes been effectively used as an indicator of the preferred degradative in in vivo pathways.

Table 8.   The logarithm of the octanol/water partition coefficients (K_{ow}) of some PCDFs using HPLC methods

PCDF	log K_{ow}	Reference
2,8-dichloro-	5.95	Sarna et al. 1984[a]
	5.30[b]	Burkhard & Kuehl, 1986[c]
2,3,7,8-tetrachloro-	5.82±0.02	Burkhard & Kuehl, 1986[c]
octachloro-	13.37	Sarna et al. 1984[a]
	8.78	Burkhard & Kuehl, 1986[c]

[a]   Quadratic equation treatment: Biorad Biosil (10 mm) data.
[b]   Quadratic equation treatment: unspecified "microbore" HPLC column.
[c]   Sarna et al. (1984) data recalculated from experimental data.

2.3.   Analytical Methods

2.3.1   General aspects

The earliest reported method used to detect 2,3,7,8-tetraCDD was a rabbit skin test (Adams et al., 1941). Test samples were applied to the inner surface of the ear and to the shaven belly of albino rabbits, and inflammatory responses were observed. Subsequently, Jones & Krizek (1962) developed a test based on the recovery and weight of the keratin formed on the rabbit ear after application of a sample. These biological methods were non-specific as to isomers and not sufficiently sensitive to detect low levels of contamination.

In the late 1960s and early 1970s, gas chromatographic methods were used for the quantification mainly of 2,3,7,8-tetraCDD in commercial 2,4,5-T formations. The detection level was normally in the range of µg/g. These analyses were not isomer-specific and the results could not be confirmed. Ryhage (1964) solved the problem of combining a gas chromatograph with a mass spectrometer. During the 1970s and 1980s, various types of mass spectrometer and gas chromatograph/mass spectrometer combinations were used in analytical work. Use of these more sophisticated instruments allowed for the development of

isomer-specific and validated analyses for the tetraCDDs in the very late 1970s and for the other PCDDs and PCDFs in the early 1980s.

A number of spectroscopic methods are available for the laboratory identification of 2,3,7,8-tetraCDD, but their use is highly restricted, with the exception of mass spectroscopy (MS). Data on X-ray, infra-red (IR), ultra-violet (UV), nuclear magnetic resonance (NMR), electron spin resonance (ESR), and mass spectra were obtained by Pohland & Yang (1972), Baughman (1974), and Slonecker et al. (1983).

Because of the large number of isomers and congeners, and due to the extreme toxicity of some PCDD and PCDF isomers, highly sensitive and specific analytical techniques are required for the measurements. Detection limits for the analysis of environmental and human samples should be orders of magnitude lower than the usual detection levels required for pesticide analysis. A detection level of 1 pg or less might be required to measure 2,3,7,8-tetraCDD and the other toxic isomers in a 1-g environmental sample. Analyses at such low levels are complicated by the presence of a multitude of other interfering compounds and clean-up procedures are required.

The mono-, di-, and trichloro congeners are not usually included in these analyses. Such compounds are considered to be much less toxic than the higher chlorinated congeners and are also much more volatile and losses may occur during clean-up.

It should be mentioned that the level of sophistication needed in the analyses for PCDD and PCDFs will depend upon the objectives thereof. In cases where the objectives were primarily to screen samples to identify groups of PCDDs and/or PCDFs (in a qualitative or semiquantitative manner), routine assays and bioassays were adequate. In other instances, where the objective of the analysis was to quantify accurately specific PCDD and/or PCDF isomers in the samples, sophisticated analytical procedures were required. Clearly, both types of analyses can be useful, depending on the purpose for which the analytical results are to be used.

Many analytical methods have been developed in recent years for the analysis of trace amounts of PCDDs and PCDFs in environmental samples, especially for 2,3,7,8-tetraCDD. The most specific of these methods are based on MS. There are many requirements to be met by such an analytical method, including representative sampling and appropriate storage, efficient extraction, high selectivity in the clean-up, high specificity in the gas chromatography, high sensitivity in the detection, safe and reliable quantification, good

reproducibility, useful confirmatory information.

Several review articles discussing methods of analyzing PCDDs and PCDFs have appeared (McKinney, 1978; Esposito et al., 1980; Rappe & Buser, 1980; Harless & Lewis, 1982; Karasek & Anuska, 1982; Tiernan, 1983; Crummett et al., 1985). Most of the older methods have been critically reviewed by a panel of experts assembled by the National Research Council of Canada (1981).

2.3.2 Sampling strategy and sampling methods

The quality and utility of analytical data depend on the validity of the sample and the adequacy of the sampling program. The purpose of sampling is to obtain specimens that represent the situation being studied. Sampling plans may require that systematic samples be obtained at specified times and places, or simple random sampling may be necessary. Generally, the sample should be an unbiased representation of the environmental situation.

All aspects of a sampling programme should be planned and documented in detail, and the expected relationship of the sampling protocol to the analytical result should be defined. A sampling programme should include reasons for choosing sampling sites, the number and type of samples, the timing of sample acquisition and the sampling equipment used. A detailed sampling procedure should include a description of the sampling situation, the sampling methodology, labelling of samples, field blank preparation, pretreatment procedures, and transportation and storage procedures.

The quality assurance programme should include means to demonstrate that containers or storage procedures do not alter the qualitative or quantitative composition of the sample. Special transportation and storage procedures (refrigeration or exclusion of light) should be described, if they are required.

Because environmental samples are typically heterogeneous, a sufficiently large number of samples (ten or more) must normally be analyzed to obtain meaningful data on chemical composition. The number of individual samples that should be analyzed will depend on the kind of information required by the investigation. If an average compositional value is required, a number of randomly selected individual samples may be obtained, combined, and blended to provide a homogeneous composite sample from which a sufficient number of subsamples could be analyzed. If composition profiles, time trends, or the variability of the sample population are of interest, many samples need to be collected and analyzed individually.

If field blanks are not available, efforts should be made to obtain blank samples that best simulate a sample that does not contain the specific chemical. In addition, measurements should be made to ascertain whether, and to what extent, any reagent or solvent used may contribute to or interfere with the analytical results (laboratory and solvent blanks).

The recovery tests are frequently used and necessary to evaluate the analytical methodology. Uncontaminated samples from control sites that have been spiked with the chemical of interest provide the best information because they simulate any matrix effect. When feasible, isotopically labelled (^{13}C, ^{37}Cl) chemicals spiked into the sample provide the greatest accuracy since they are subjected to the same matrix effects. The ^{13}C- and ^{37}Cl-labelled compounds can be used to validate:

(a) sampling (sampling surrogate),
(b) analytical pretreatment (clean-up surrogate),
(c) quantification (internal standard).

Very few laboratories in the world have access to and experience in working with these complicated analyses.

In order to be able to compare data generated in different laboratories, the same quantitative standard compounds should be used. Interlaboratory calibrations or "round-robin" studies have been performed in very few cases.

2.3.3 Extraction procedures

In this step, the sample is homogenized or digested and extracted with a suitable solvent or solvent mixture to remove the bulk of the sample matrix and transfer the PCDD and PCDF residue into the solvent. Both the selection of the proper solvent and the method of extraction can be critical in obtaining a satisfactory recovery of PCDDs and PCDFs from the sample matrix.

Many different procedures for the extraction of PCDDs/PCDFs from various samples are described. In some cases this involves digestion or destruction of the matrix. Some of these methods have been evaluated in the report from the National Research Council of Canada (1982), while other methods are discussed by Tiernan (1983).

An interlaboratory "round-robin" study involving 13 laboratories was carried out to evaluate the reliability of data on

2,3,7,8-tetraCDD in fish samples. No significant differences were
found from methods differing in the digestion or extraction procedures
(Ryan et al., 1983b).

      In a study described by Albro et al. (1985), eight different
approaches were applied in eight laboratories to quantify four PCDDs
(2,3,7,8-tetraCDD; 1,2,3,7,8-pentaCDD; 1,2,3,4,7,8-hexaCDD; and
octaCDD) and three PCDFs (2,3,7,8-tetraCDF; 2,3,4,7,8-pentaCDF; and
1,2,3,7,8,9-hexaCDF) in spiked samples of an extract from human
adipose tissue. Levels of fortification, unknown to the participating
laboratories, were in the 5-50 ng/kg range, except for octaCDD (up to

500 ng/kg). The results indicated that most of the procedures tested
gave a high degree of qualitative reliability. However, other methods
were not so accurate, a large portion of the reported data consisting
of false positives or false negatives.

      Lustenhouwer et al. (1980) studied the extraction of PCDDs and
PCDFs from a fly ash sample. A dramatic difference was found between
different solvents.

2.3.4 Sample clean-up

      In the sample clean-up, the PCDDs and PCDFs present in the sample
should be separated from a multitude of other co-extracted and
possibly interfering compounds. The clean-up methods, normally three
steps or more, can vary for different sample matrices. Two different
procedural trends can be recognized:

      (a)   all PCDD and PCDF isomers can be analyzed in one
            single fraction by the containment enrichment
            procedure (Norstrom et al., 1982; Stalling et al.,
            1983; Tiernan, 1983; Rappe, 1984),

      (b)   specific isomers are analyzed in different fractions
            mainly after normal-phase and reverse-phase high
            pressure liquid chromatography (HPLC) separation
            (Lamparski et al., 1979; Niemann et al., 1983; Tosine
            et al., 1983).

      This latter method allows the identification of only a few PCDD
isomers in each fraction, and is mainly used to monitor TCDD and a few
other congeners. For a monitoring program of all PCDDs and PCDFs a
more general method might be preferred.

      The method described by Stalling et al. (1983) was originally

designed for the analyses of fish samples. In a "round-robin" study of fish samples it gave good results (Ryan et al., 1983b). This method has now been used for the clean-up of other biological samples like bird muscle, seal fat, turtle fat, and human adipose tissue - blood, liver, kidney, and milk (Rappe et al., 1983c; Nygren et al., 1986; Rappe et al., 1986b).

2.3.5 Isomer identification

The purified extracts are used directly for the final analyses with the aid of a gas-chromatograph/mass spectrometer (GC/MS) equipped with a glass capillary or a fused-silica column. The column leads directly into the ion source of the mass spectrometer, which operates either in the electron impact (EI) or the negative ion-chemical ionization (NCI) mode. In view of the large variation in toxicological

and biological effects of the PCDD and PCDF isomers, it is imperative that the isomers, particularly those having high toxicity, be identified. For an unambiguous isomer identification it is necessary to have access to all analytical standards within a specific group of isomers, e.g. all the 22 tetraCDDs and all the 38 tetraCDFs. All the 22 tetraCDDs have been prepared and, using a Silar 10c glass capillary column, the 2,3,7,8-tetraCDD can be separated from all the other 21 tetra isomers (Buser & Rappe, 1980). Recently all the 14 pentaCDDs and the 10 hexaCDDs have been prepared. Using the Silar 10c column all the 2,3,7,8- substituted isomers can be separated from all the other isomers (Buser & Rappe, 1984). The SP 2330 fused silica column can also be used for this separation (Rappe, 1984).

In the PCDF series, Mazer et al. (1983) have synthesized all the 38 positional tetraCDF isomers. The products were mixtures of isomers, and each of these isomers could be identified using both an SP 2330 and an SE 54 capillary column. Later, Bell & Gara (1985) isolated and characterized all tetra-, penta- and hexaCDFs. The SP 2330 column can separate most of these isomers (Rappe, 1984). The 1,2,3,7,8-pentaCDF co-elutes with the 1,2,3,4,8-isomer and the 1,2,3,4,7,8- hexaCDF with the 1,2,3,4,7,9-isomer, but they can be separated on less polar columns like OV-17 and DB-5.

A very limited number of investigations has been performed using these complete sets of synthetic standards.

2.3.6 Quantification

Mass selective detection (mass fragmentography) has been used to quantify trace amounts of PCDDs and PCDFs in the samples by

selectively monitoring M, M + 2, and/or M + 4 ions (SIM). The quantification is based on peak area measurements and a comparison of these areas using either isotopically labelled internal standards (^{13}C or ^{37}Cl) or calibration curves of external standards. As a first approach, it has been generally assumed that with the MS quantification technique, all isomers of a particular congener of PCDD or PCDF (e.g. the tetrachloro-isomers) have the same response factors. However, an investigation of 13 well-defined tetraCDF isomers has shown a three-fold variation in response factors with the EI mode and up to a 20-fold variation with the negative ion-chemical ionization mode. For the higher chlorinated homologues (penta, hexa) the variation was found to be less (Rappe et al., 1983b).

Fung et al. (1985) have studied the mass spectra of 26 PCDF congeners. They found that the EI spectra are not particularly isomer specific, while positive ion-chemical ionization spectra show a greater degree of isomer distinction.

2.3.7 Confirmation

Quality control and quality assurance programs help to assure that positive data reported actually refer to specific PCDDs and PCDFs (Kloepfer et al., 1983). To provide reliable data:

(a) isomer specificity must be demonstrated initially and verified daily,
(b) the retention time must equal (within 3 seconds) the retention time for the isotopically labelled congener,
(c) the signal to noise ratio must be 2.5:1 or higher,
(d) the chlorine cluster must be within ± 10% of the theoretical values, given in Table 9,
(e) correct fragments, e.g., M^+-COCl ions, must be with correct chlorine clusters.

For confirmation, mass spectroscopy is the best technique now available. The EI mass spectral properties of PCDFs and PCDD have been described (Buser, 1975). The molecular (M^+) and fragment ions of PCDDs and PCDFs show the typical, expected clustering due to the chlorine isotopes (Table 9). The typical fragmentation is M^-COCl$^+$, which is a useful fragment to study.

Buser & Rappe (1978) have shown that observation of low mass ions can be used for the identification of the substitution pattern of PCDDs, which can be defined as the number of chlorine atoms on each carbon ring of the dioxin molecular; the 2,3,7,8-isomer has a 2:2 pattern while 1,2,3,4-tetraCDD has a 4:0 pattern. However, these low

mass ions may not be observed in spectra from environmental or
biological samples.

In the negative ion-chemical ionization mode, the PCDFs have the
base peak due to M^-, and the fragmentation produces the unusual
M^--34 ions (uptake of H and loss of Cl). Fragmentation of PCDDs in
this mode is more conventional via loss of Cl yielding M^--35 ions
(Buser et al., 1985).

Using EI technique and a quadropole instrument, the detection
limits are 1-10 pg for the tetrachloro compounds and up to 10-50 pg
for the octachloro compounds using selected ion monitoring or multiple
ion detection (SIM or MID). Full mass spectra require 0.1-1 ng of
compound (Buser et al., 1985). High resolution instruments can improve
the sensitivity by one order of magnitude.

The negative ion-chemical ionization mode, using methane gas as
reagent, gas provides extremely good sensitivity for all PCDFs (tetra-
to octachloro- compounds) and for the higher chlorinated PCDDs (penta-
to octaCDD). The detection limits are in the 10-100 fg (10^{-15}g)
range using SIR or MID, which is 1 to 2 orders of magnitude better
than EI (Buser et al., 1985). However, the negative ion-chemical
ionization mode has very poor sensitivity for 2,3,7,8- tetraCDD under
these conditions.

Using low resolution MS instruments, a series of interfering
compounds has been identified (Table 10). Some of this interference
can be eliminated using high resolution MS instruments operating at
8000 - 10 000 daltons. However, compounds with the same empirical
formulae cannot be separated by MS technique; they are normally
eliminated during the clean-up or separated by the gas chromatography
step.

2.3.8 Other analytical methods

Paasivirta et al. (1977) have shown that 2,3,7,8-tetraCDD can be
detected down to the pg level using a glass capillary column and a
[63]Ni electron-capture detector. Combined with efficient clean-up
procedures, this method has shown to be useful down to a level of 9
ppt (Niemann et al., 1983), although positive samples need
confirmation by mass spectroscopy (MID, SIM).

Other techniques, such as enzyme induction and radioimmunoassay
have been described and discussed by Firestone (1978) and McKinney
(1978). McKinney et al. (1982) have used the radioimmunoassay method

for determining 2,3,7,8-tetraCDD in human fat, and found the reliable sensitivity at 95% confidence interval to be 100 pg per sample.

An analytical method based on the keratonization response of epithelial cells in an in vitro system has been described by Gierthy & Crane (1985b). This method can be an assay for dioxin-like activity in environmental and biological samples. A positive response was found for 2,3,7,8-tetraCDD at a concentration of 10-11 mol/litre.

Table 9. Isotopic abundance ratio ("cluster") of polychlorinated dioxins and dibenzofurans

Number of chlorine atoms	M	M + 2	M + 4	M + 6	M + 8	M + 10	M + 12	M + 14
1	100.0	33.7						
2	100.0	66.1	11.3					
3	100.0	98.4	32.7	3.8				
4	76.4	100.0	49.4	11.0	1.0			
5	61.2	100.0	65.5	21.6	3.6	0.3		
6	51.1	100.0	81.7	35.8	8.9	1.2	0.1	
7	43.8	100.0	97.9	53.4	17.6	3.5	0.4	
8	33.7	87.6	100.0	65.3	26.8	7.0	1.2	0.1

Table 10. List of molecular ions of polychlorinated compounds present in some human and environmental samples and possibly interfering in the mass spectral analysis of PCDFs and PCDDs[a]

Molecular ions (m/z,m$^+$, m$^-$) (chlorination)

Compounds	mono-	di-	tri-	tetra-	penta-	hexa-	hepta-	octa-	nona-	deca-
PCDDs				320	354	388	422	456	–	–
PCDFs				304	338	372	406	440	–	–
PCBs				290	324	358	392	426	460	494
PCNs				264	298	332	366	400	–	–
PCTs		298	332	366	400	434	468	502	536	570
PCDPEs[b]		238	272	306	340	374	408	442	476	510
PCPYs[c]	36	270	304	338	372	406				

[a]   From: Buser et al. (1985).

[b]   PCDPEs: Polychlorinated diphenylethers.

[c]   PCPYs: Polychlorinated pyrenes.

3.   SOURCES OF ENVIRONMENTAL POLLUTION

3.1 Production, Synthesis, and Use

PCDDs and PCDFs are not produced commercially. These compounds are in fact formed as trace amounts of undesired impurities in the manufacture of other chemicals such as chlorinated phenols and their derivatives, chlorinated diphenyl ethers, and polychlorinated biphenyls (PCBs). There is no known technical use for the PCDDs and PCDFs.

The amount of total PCDDs entering the Canadian environment/year has been estimated to be about 1500 kg, and 75% of this amount has been estimated to be due to octaCDD alone (National Research Council of Canada, 1981). There is no estimation of the amount of PCDFs entering the environment anywhere in the world.

Although the polychlorinated dioxins and dibenzofurans are not
commercially produced, most of these compounds have been synthesized
for research purposes in small quantities according to the reactions
discussed in section 2.

3.2   Industrial Processes

In addition to the synthetic methods mentioned in section 2,
2,3,7,8-tetraCDD may be formed during the industrial preparation of
2,4,5-trichlorophenol from 1,2,4,5-tetra-chlorobenzene. This
substitution reaction takes place at about 180 °C, and when the
solvent is methanol, the pressure rises to about 7 KPa. The formation
of TCDD is an unwanted side reaction which takes place when the
reaction mixture is heated to 230-260 °C (Milnes, 1971). This reaction
is exothermic, so that even higher temperatures may be attained
resulting in uncontrolled conditions.

In some factories ethylene glycol is used as a solvent in order
to avoid the high pressure. As already pointed out by Milnes (1971),
however, use of this solvent requires special precautions because of
the occurrence of a base-promoted polymerization of ethylene glycol
and decomposition reactions that produce ethylene oxide. These
reactions are also exothermic; they may start spontaneously at
temperatures above 180 °C and proceed rapidly and uncontrollably to
result in the formation of relatively large amounts of TCDD.

After most of the solvent has been removed, the reaction mixture
is acidified; the 2,4,5-trichlorophenol can be separated from
2,3,7,8-tetraCDD by one or two distillations, with the result that
2,3,7,8-tetraCDD is concentrated in the still-bottom residues. Up to
1 mg/g of 2,3,7,8-tetraCDD in such residues has been reported
(Kimbrough et al., 1984). Improper disposal of such residues is
discussed in sections 4.4.2 and 9.

Most of the 2,4,5-trichlorophenol produced is used for the
preparation of herbicides such as 2,4,5-T (including various esters
and salts, and the bactericide hexachlorophene).

PCDDs and PCDFs are both formed as by-products during the
manufacture of chlorinated phenols (2,4-dichloro-, 2,4,6-trichloro-,
2,3,4,6-tetrachloro- and pentachlorophenol). The commercial
chlorophenols are produced by two processes, i.e., by chlorination of
the phenol using various catalysts and by the alkaline hydrolysis of
an appropriate chlorobenzene. Apparently both reactions can lead to
the formation of PCDDs as well as PCDFs, and the level of
contamination is normally much higher here than in the production of

2,4,5-trichloro-phenol (see section 3.3).

PCDDs and PCDFs are also formed during the preparation of chlorinated diphenyl ether herbicides (Yamagishi et al., 1981) and hexachlorobenzene (Villeneuve et al., 1974). A series of PCDFs are formed during the production of PCBs (see section 3.3).

Production equipment is often used for the production of several different chemicals. In the manufacture of chemicals on such equipment previously contaminated by PCDDs and PCDFs, both the products and waste generated can be contaminated. Thus, manufactured 2,4-dichlorophenoxyacetic esters (2,4-D), which otherwise should not be contaminated by 2,3,7,8-tetraCDD, did indeed contain this dioxin because the equipment used had been employed previously to produce 2,4,5-T and had not been cleaned properly (Federal Register, 1980).

It should be pointed out that the primary occurrence of TCDD in the environment is possibly related to the synthesis of 2,4,5-trichlorophenol, the use of products prepared from this compound (Table 11), and to incinerations reactions. The occurrence of the other PCDDs and PCDFs is related to the synthesis and use of a variety of other products (Table 12), some of which are quite common.

The other PCDDs and PCDFs are also formed in a variety of incineration reactions (see section 4.5).

3.3 Contamination of Commercial Products

3.3.1 Chlorophenoxyacetic acid herbicides

Depending on the temperature control and purification efficiency, the levels of 2,3,7,8-tetraCDD in commercial products may vary greatly. For example, the levels of 2,3,7,8-tetraCDD in drums of the herbicide Agent Orange placed in storage in the USA and in the Pacific

before 1970 were between 0.02 and 47 mg/g. More than 450 samples were analyzed in this study, and the mean value was 1.98 mg/g (Young et al., 1983). Since Agent Orange was formulated as a 1:1 mixture of the butyl esters of 2,4,5-T and 2,4-D, the levels of 2,3,7,8-tetraCDD in individual 2,4,5-T preparations manufactured and used in the 1960s could have been as high as 100 mg/g.

In analyses using high-resolution GC-MS, Rappe et al. (1978a) have reported that in other samples of Agent Orange (as well as in European and the USA 2,4,5-T formulations from the 1950s and 1960s), 2,3,7,8-tetraCDD was the dominating compound of this group of

contaminants. Only minor amounts of other PCDDs and PCDFs could be found, primarily lower chlorinated PCDDs, in samples of Agent Orange.

As a result of governmental regulations, efforts were made during the 1970s to minimize the formation of 2,3,7,8-tetraCDD during 2,4,5-T production, and now all producers claim that their products contain less than 0.1 µg 2,3,7,8-tetraCDD/g of product (Rappe et al., 1978a). At present, the chloro-phenoxy herbicides are not the major source of PCDDs and PCDFs in the environment.

Sixteen samples of 2,4-D esters and amine salts from Canada were analyzed for the presence of PCDDs. Eight out of nine esters and four out of seven amine salts were found to be contaminated, with the esters showing significantly higher levels (210-1752 ng/g) than the salts (20-278 ng/g). The tetraCDD observed was the 1,3,6,8-isomer, as verified by a synthetically prepared authentic standard (Cochrane et al., 1981). In other studies, it has been found that no tetraCDD other than the 1,3,6,8-isomer elutes in this window. Hagenmaier et al. (1986) has reported that, unexpectedly, a German 2,4-D formulation contained 6.8 ng of 2,3,7,8-tetraCDD/g.

Table 11. Some commercial products that may be contaminated with 2,3,7,8-tetraCDD, depending on the method of preparation

Common name	Chemical name
2,4,5-T[a]	2,4,5-Trichlorophenoxyacetic acid
2,4,5-T esters[a]	n-butyl-, butoxy ethyl-, and iso-octyl-esters of 2,4,5-trichlorophenoxyacetic acid
2,4,5-T salts[a]	dimethylamine salts of 2,4,5-trichlorophenoxyacetic acid
Fenoprop	esters of 2-(2,4,5-trichlorophenoxy)-propanoic acid
Erbon	ethyl ester of 2-(2,4,5-trichloro-phenoxy)-2,2-dichloropropanoic acid

```
2,4,5-Trichlorophenol            2,4,5-Trichlorophenol

Fenochlorphos                    O,O-Dimethyl O-2,4,5-trichlorophenyl
                                 phosphonothioate


Trichloronate                    O-Ethyl 0-2,4,5-trichlorophenyl
                                 ethylphosphonothioate


Hexachlorophene/isobac 20        2,2'-Methylene-bis (3,4,6-trichloro-
                                 phenol)
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a   There are numerous trade names for this product.

Table 12. Some commercial products which may be contaminated with PCDDs
other than 2,3,7,8-tetraCDD, and with PCDFs, depending on the method of
preparation

Common name	Chemical name
Bifenox	Methyl-5-2,4-dichlorophenoxy-2-nitrobenzoate
Chloranil	2,3,5,6-Tetrachloro-2, 5-cyclo-hexadiene-1,4-dione.
2,4-D (esters and salts)	2,4-Dichlorophenoxyacetic acid and esters and salts
2,4-DB and salts	2,4-Dichlorophenoxybutyric acid and salts
Dicamba	3,6-Dichloro-2-methoxybenzoic acid
Dicamba, dimethylamine salt	3,6-Dichloro-2-methoxybenzoic acid, dimethylamine salt
Dicapthon	Phosphorothioic acid o-(2-chloro-4-nitrophenyl) o,o-dimethyl ester
Dichlofenthion	Phosphorothioic acid o-2,4-dichloro-phenyl o,o-dialkyl ester
Disul sodium (sesone)	2,4-Dichlorophenoxyethyl sulfate, sodium salt

2,4-DP	2- 2,4-Dichlorophenoxy propionic acid
HCB	Hexachlorobenzene
Nitrofen	2,4-Dichlorophenyl-p-nitrophenyl ether
PCP and salts	Pentachlorophenol and salts
PCB	Polychlorinated biphenyls
2,4,6-TCP	2,4,6-Trichlorophenol and salts 2,3,4,6-Tetrachlorophenol and salts

Common name	Chemical name
CNP	1,3,5-Trichloro-2-(4-nitrophenoxy) benzene
NIP	2,4-Dichloro-1-(4-nitrophenoxy) benzene
X-52	2,4-Dichloro-1-(3-methoxy-4-nitro-phenoxy) benzene

3.3.2 Hexachlorophene

The bactericide hexachlorophene is prepared from
2,4,5-trichlorophenol, also the key intermediate in the production of
2,4,5-T. Due to additional purification, the level of 2,3,7,8-tetraCDD
in this product is usually < 0.03 mg/kg (Baughman, 1974). Ligon & May
(1986) reported 0.0047 mg/kg of TCDD in one hexachlorophene sample.
However, hexachlorophene also contains about 100 mg/kg of a
hexachloroxanthene, the 1,2,4,6,8,9-substituted isomer (Göthe &
Wachtmeister, 1972).

3.3.3 Chlorophenols

Chlorophenols have been used extensively since the 1950s as
insecticides, fungicides, mold inhibitors, antiseptics, and
disinfectants. In 1978 the annual world production was estimated to be

approximately 200 000 tons. The most important use of 2,4,6-tri-, 2,3,4,6-tetra-, and pentachlorophenol, and their salts, is for wood preservation. Pentachlorophenol is also used as a fungicide for slime control in the manufacture of paper pulp and for a variety of other purposes such as in cutting oils and fluids, for tanning leather, and in paint, glues, and outdoor textiles. 2,4-Di- and 2,4,5-trichloro-phenol are used for the production of 2,4-D and 2,4,5-T herbicides (phenoxy acids), and 2,4,5-trichlorophenol for the production of hexachlorophene.

Chlorophenols are produced industrially either by direct chlorination of phenol or by hydrolysis of chlorobenzenes, the actual process used depending on the isomer desired. Chlorination of phenol yields 2,4-di-, 2,4,6-tri-, 2,3,4,6-tetra-, or pentachlorophenol, while hydrolysis of chlorobenzenes is mainly used for the production of 2,4,5-tri- and pentachlorophenol (Nilsson et al., 1978). Chlorophenols may contain a variety of by-products and contaminants, such as other chlorophenols, polychlorinated phenoxyphenols, and neutral compounds like polychlorinated benzene and diphenyl ethers

(PCDPEs), PCDDs, and PCDFs. Some of these contaminants may also occur in chlorophenol derivatives like phenoxy acids, other pesticides, and hexachlorophene. The possible presence of PCDDs and PCDFs in commercial products is of special significance because of their extraordinary persistence and toxicological properties (see sections 7-9). A scientific criteria document for chlorophenols and their impurities in the Canadian environment has been prepared by Jones (1981, 1984). Chlorophenols were estimated to be the major chemical sources of PCDDs and PCDFs in the Canadian environment (Sheffield, 1985).

Buser & Bosshardt (1976) reported on the results of a survey of the PCDD and PCDF contents of pentachlorophenol (PCP) and PCP-Na from commercial sources in Switzerland. From the results, a grouping of the samples into two series can be observed: a first series with generally low levels (hexaCDD <1 µg/g) and a second series with much higher levels (hexaCDD >1 µg/g) of PCDDs and PCDFs. Samples with high PCDD values had also high PCDF values. For most samples, the contents of the PCDF contaminants were in the order:

tetra- = penta- < hexa- < hepta- < octaCDD/CDF.

The ranges of the combined levels of PCDDs and PCDFs were 2-16 and 1-26 µg/g, respectively, for the first series of samples, and 120-500 and 85-570 µg/g, respectively, for the second series of samples. The levels of octaCDD and octaCDF were as high as 370 and 300 µg/g,

respectively.

Some PCP-Na samples analyzed showed the unexpected presence of a tetraCDD (0.05-0.25 µg/g), which was later identified by Buser & Rappe (1978) as the unusual 1,2,3,4-substituted isomer. Table 13 collects a number of relevant analyses of these chlorophenol formulations. The levels of PCDDs and PCDFs are higher than for the phenoxy-acetic acid herbicides.

It has also been reported that several positional isomers of PCDDs and PCDFs are present in the chlorophenols. However, isomer-specific methods have not been used in most of these investigations, and more research is necessary to identify all the isomers present for a risk evaluation of these products.

Miles et al. (1985) have analyzed PCP samples for hexaCDDs from five different manufacturers using an isomer-specific analytical method. The study included both free PCPs as well as the sodium salts. Total hexaCDDs in PCPs ranged from 0.66 to 38.5 mg/kg, while in the sodium salts levels of hexaCDDs between 1.55 and 16.3 mg/kg were found. The most abundant hexaCDD isomer found in the free PCPs was the 1,2,3,6,7,8 isomer; however, in the sodium salts the 1,2,3,6,7,9- and 1,2,3,6,8,9-hexaCDD pair was the most abundant.

Table 13. Levels of PCDDs and PCDFs in commercial chlorophenols (µg/g)[a]

	2,4,6-Trichlorophenol	2,3,4,6-Tetrachlorophenol	PCP Sample A	PCP Sample B
TetraCDDs	< 0.1	< 0.1	< 0.1	< 0.1
PentaCDDs	< 0.1	< 0.1	< 0.1	< 0.1
HexaCDDs	< 1	< 1	< 1	2.5
HeptaCDDs	< 1	10	0.5	175
OctaCDD	< 1	2	4.3	500
TetraCDFs	1.5	0.5	< 0.1	< 0.1
PentaCDFs	17.5	10	< 0.1	< 0.1
HexaCDFs	36	70	0.03	< 0.3
HeptaCDFs	4.8	70	0.5	19
OctaCDF	< 1	10	1.1	25

[a]   From: Rappe et al. (1979).

     Hagenmaier & Brunner (1987) has reported that 2,3,7,8-tetraCDD
can be found in commercial pentachlorphenol formulation at levels of
0.21-0.56 ng/g, while Hagenmeyer & Brunner (1986) report that
1,2,3,7,8-pentaCDD was found in pentachlorophenol and
Na-pentachlorophenates in concentrations of 0.9-18 ng/g.

3.3.4 Polychlorinated biphenyls (PCBs)

     Vos et al. (1970) were able to identify PCDFs (tetra- and
pentaCDFs) in samples of European PCBs (Phenoclor DP-6 and Clophen A
60) but not in a sample of Aroclor 1260. The toxic effects of these
PCB products were found to parallel the levels of PCDFs present. Bowes
et al. (1975) examined a series of Aroclors, as well as the samples of
Aroclor 1260, Phenoclor DP-6, and Clophen A-60 previously analyzed by
Vos et al. (1970). They used packed columns and very few standard
compounds, and reported that the most abundant PCDFs had the same
retention time as 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF. Using a
complete set of PCDF standards and an isomer-specific analytical
method, Rappe et al. (1985d) determined the levels of
2,3,7,8-substituted PCDFs in commercial PCB products (see Table 14).

3.3.5 Chlorodiphenyl ether herbicides

     In 1981, Yamagishi et al. reported on the occurrence of PCDDs and
PCDFs in the commercial diphenyl ether herbicides
1,3,5-trichloro-2-(4-nitrophenoxy) benzene (CNP),
2,4-di-chloro-1-(4-nitrophenoxy)benzene (NIP), and
2,4-dichloro-1-(3-methoxy-4-nitrophenoxy)benzene (X-52). The total
tetraCDD found was 14.0 mg/kg in CNP, 0.38 mg/kg in NIP, and 0.03 in
X-52. Very few synthetic standards were used, but the major tetraCDDs
were identified as the 1,3,6,8- and 1,3,7,9-isomers, the expected
impurities in the starting material 2,4,6-trichlorophenol. No
2,3,7,8-tetraCDD could be detected in these samples. In all three
herbicides, total tetraCDF was between 0.3 and 0.4 mg/kg.

3.3.6 Hexachlorobenzene

     Hexachlorobenzene was used for the control of wheat bunt and
fungi. Villeneuve et al. (1974), analyzing three commercial
hexachlorobenzene preparations, identified octaCDD and hepta- and
octaCDFs. The levels and identity of the heptaCDF isomers were not
given. Great variation in levels of octaCDDs between the three samples
(0.05-211.9 mg/kg) was noted, as well as in the level of octaCDF
(0.35-58.3 mg/kg).

3.3.7 Rice oil

     In 1968 more than 1500 people in southwest Japan were intoxicated
by the consumption of a commercial rice oil accidentally contaminated
by PCBs, PCDFs, and polychlorinated quarterphenyls (Masuda &
Yoshimura, 1982; Masuda et al., 1985). In 1979 a similar episode
occurred in central Taiwan, the number of people involved here
approaching 2000 (Chen et al., 1980, 1981). Both these accidents have
been referred to as Yusho episodes, but now the Taiwan episode has
been renamed Yu-cheng (see section 5.4.4.4).

     The total level of PCDFs in the Japanese rice oil was reported to
be 5 µg/g (Nagayama et al., 1976) and 5.6 µg/g (Buser et al., 1978d).
For the rice oil from Taiwan, Chen et al. (1985) reported the PCDFs
levels to be in the range 0.18-1.68 µg/g.

     Buser et al. (1978) analyzed the Japanese rice oil using glass
capillary columns. They found about 50-60 PCDF congeners and also
reported that the 2,3,7,8-tetraCDF was the major isomer among the
tetraCDFs. However, it was later shown that in this column system the
2,3,4,8-tetraCDF co-elutes with the 2,3,7,8-isomer, and in fact the
2,3,4,8-isomer was the main constituent in this peak (Chen & Hites,
1983; Masuda et al., 1985). The 2,3,7,8-substituted congeners were
estimated to account for 10-15% of the total amount of PCDFs (Buser et
al., 1978).

Table 14. PCDFs in commercial PCBs (ng/g)[a]

```
                     TRI-
TETRA-               PENTA-                      HEXA-                   HEPTA-
       _____        _____         _____        _____  _____

                     Total    2378  Total  12348  23478  Total
123479 123678 123789 234678 Total  Total
       PCB-type                            12378                 123478


       Pyralene        700     53     630    10     T      35
ND     ND     ND       ND            ND     ND
       A1254            63     19    1400   690    490   4000
2500   2100   190      130    10 000  960
```

	A1260			10	13	110	48	56	260
500	120	190	27	1500	1300				
	A30		500	35	573	14	28	160	
50	59	ND	ND	220	T				
	A40		1300	180	2600	96	8	1700	
79	68	ND	T	310	ND				
	A50		7400	3300	20 000	760	1100	8000	
700	360	18	98	3100	75				
	A60		770	840	6900	1100	990	8100	
1600	330	170	330	6800	2000				
	T64		47	23	360	97	122	840	
520	390	58	41	2600	220				
	Clophen C		710	54	1200	34	30	270	
ND	T	ND	ND	T	ND				

a From: Rappe et al. (1985d).
T = traces.
ND = not detected.

3.4 Sources of Heavy Environmental Pollution

3.4.1 Industrial accidents

Several industrial accidents occurring during the production of 2,4,5-trichlorophenol have been described in the literature. In most of these accidents the pollution of 2,3,7,8-tetraCDD has been to factories with circumscribed occupational exposure (section 9). However, on 10 July, 1976, a runaway reaction in a factory at Meda near Seveso in Northern Italy resulted in the escape of a chemical cloud of trichlorophenol/phenate containing 2,3,7,8-tetraCDD.

The cloud initially covered an area outside the factory 5 km long and 700 m wide. On the basis of the TCDD levels found in the contaminated soil samples, it has been estimated that 2-3 kg of TCDD was released in this accident. About 80% of this amount was deposited in an area of 15 ha, within a distance of about 500 m from the plant. The levels of soil contamination in three zones are given in Table 15 (Pocchiari, 1978).

3.4.2 Improper disposal of industrial waste

In 1973, three horse arenas in Missouri, USA, were found to be contaminated by high levels of 2,3,7,8-tetraCDD; the highest value

reported was about 30 µg/g of soil (Kimbrough et al., 1977). This
contamination resulted from the application, in 1971, of contaminated
waste oil to control dust at these locations. The TCDD had originated
at a hexachlorophene-producing factory in Verona, Missouri. Additional
tri- and tetraCDDs were also found, but the major component was
1,2,4,6,8,9-hexachloroxanthene, a compound which apparently can serve
as a marker for this type of contamination. The xanthene is a normal
by-product of hexachlorophene production and has never been associated
with the production of 2,4,5-tri-chlorophenol or 2,4,5-T derivatives
(Viswanathan & Kloepfer, 1986).

In 1982, numerous sites of potential 2,3,7,8-tetraCDD
contamination were discovered in eastern Missouri. The contamination
originated from the same waste oil from the factory in Verona. The
streets of the entire town of Times Beach, Missouri, had been sprayed.
More than 10 000 soil samples from Missouri were analyzed. In this
state more than 40 hazardous waste sites containing 2,3,7,8-tetraCDD
were identified. Most of these contaminated sites resulted from the
disposal of waste from the same factory in Verona. The highest level
reported in these soil samples was 9648 mg TCDD/g (Viswanathan &
Kloepfer, 1986).

Another location of great concern is Love Canal, Niagara Falls,
USA. Here, Smith et al. (1983) found high levels of 2,3,7,8-tetraCDD
in storm sewer sediments taken from around the Love Canal waste
disposal site. The highest value was 312 ng/g sediment.

Table 15. Distribution of TCDD contamination in the Seveso area on the basis
of soil sample analyses[a]

Range ($\mu g/m^2$)	Number of soil samples		
	Zone A	Zone B	Surrounding monitored area
< 0.750	32	25	249
0.750 – 4.99	32	53	128
5.0 – 14.99	6	19	2
15.0 – 49.99	18	6	0
50.0 – 499.99	31	0	0

```
     500.0 - 4999.99          18            0                  0
     > 5000                    3            0                  0
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^a From: Pocchiari (1978).
     Zone A: high-level contamination, about 115 ha.
     Zone B: low level contamination, about 255 ha.
     Surrounding area: about 1400 ha.

3.4.3 Heavy use of chemicals

     The Eglin Air Force Base in Northwest Florida, USA, has been used
for the development and testing of aerial spraying equipment for
military defoliation operations. During the period 1962-1970, a
3-km^2 test area was sprayed with 73 tons of 2,4,5-T. Analyses of
archived samples of the formulations indicated that approximately 2.8
kg of 2,3,7,8-tetraCDD had been applied as a contaminant of the
herbicide. However, one 37-ha test grid received 2.6 kg of this TCDD
from 1962 to 1964. Levels of 10-1500 ng/kg were found in 22 soil
samples (the top 15 cm) collected and analyzed 14 years after the last
application of herbicide to this site (Young, 1983).

3.5 Other Sources of PCDDs and PCDFs in the Environment

3.5.1 Thermal degradation of technical products

     The formation of 2,3,7,8-tetraCDD as a result of thermal
reactions of 2,4,5-T and 2,4,5-T derivatives has been the subject of
controversy. Heating 2,4,5-T salts at 400-450 °C for 30 minutes or
longer yielded approximately 1 g of 2,3,7,8-tetraCDD per kg of 2,4,5-T
salt, while no TCDD was identified from the same treatment of 2,4,5-T
acid or esters (Langer et al., 1973; Baughman, 1974). Using a more
sensitive analytical method, Ahling et al. (1977) reported that 0.2-3
mg of 2,3,7,8-tetraCDD was formed per kg of 2,4,5-T esters during

combustion at 500-850 °C. Two reports (Stehl & Lamparski, 1977;
Andersson et al., 1978) have shown that 2,3,7,8-tetraCDD could not be
found after burning samples of spiked or sprayed vegetation at 600 °C.
The combustion gases, soot, particles, and ashes were analyzed and the
detection limit was 4 mg of TCDD/kg 2,4,5-T burned.

     Rappe (1978b) have studied the burning of material impregnated
with various salts of chlorophenols. Very carefully purified
2,4,6-tri- and pentachlorophenate were studied, in addition to a

commercial formulation of 2,3,4,6-tetra-chlorophenate. The analytical method used in this study was not isomer specific, but the following conclusions can be drawn concerning the formation of PCDDs by thermal reactions:

(a) the expected dimerization products and the products formed in the "Smiles rearrangement" are the major PCDDs;

(b) no other thermal isomerization of the PCDDs formed can be observed;

(c) no formation of higher chlorinated PCDDs can be observed;

(d) octaCDD and other higher chlorinated PCDDs yield lower chlorinated dioxins in a nonspecific dechlorination reaction;

(e) a series of PCDFs was also observed.

It has been found that PCBs can be converted to PCDFs under pyrolytic conditions. The pyrolysis of commercial PCBs in sealed quartz ampoules in the presence of air yielded about 30 major, and more than 30 minor, PCDFs. The optimal yield of PCDFs was about 10%, calculated on the amount of PCB decomposed. Thus, uncontrolled burning of PCBs can be an important environmental source of hazardous PCDFs. Therefore, it was recommended (Buser et al., 1978a, 1978d) that all destruction of PCB-contaminated waste using incinerators must be carefully controlled. In the temperature range 300-400 °C, the conversion yield seems to be in the part-per-million range (Morita et al., 1978).

Buser & Rappe (1979) studied the pyrolysis of 15 individual synthetic PCB congeners and showed that the formation of PCDFs can follow several competing reaction pathways. In another study where a series of chlorobenzenes were pyrolyzed in the same way, Buser (1979) found that significant amounts (> 1%) of PCDDs and PCDFs were formed. A complex mixture of isomers of PCDDs and PCDFs was found, suggesting several reaction routes. Using the same technique as above, Lindahl et al. (1980) studied the thermal decomposition of polychlorinated diphenyl ethers. Both PCDDs and PCDFs were formed, involving several pathways. The temperature range was 500-600 °C and the yields varied from 0.1 to 4.5%.

Bergman et al. (1984) studied the thermal degradation of two polychlorinated alkanes containing 59% and 70% chlorine, respectively, and also a commercial chlorinated paraffin containing 70% chlorine. Their studies indicated the presence of at least mono- and diCDFs.

Ahling et al. (1978) reported that chlorinated benzenes can be found in the pyrolysis of PVC.

Direct evidence for the conversion of PVC to PCDDs and PCDFs has
recently been reported by Marklund et al. (1986). They found that
laboratory pyrolysis of PVC resulted in the formation of PCDDs and
PCDFs, mainly hexa- and heptaCDDs, and tetra- to heptaCDFs. In some
cases, the pattern of isomers seemed to be similar to those found in
municipal and hazardous waste incinerators, e.g. the pentaCDFs (Rappe
et al., 1987).

The data discussed in this section are summarized in Table 16.

3.5.2 Incineration of municipal waste

For some time, emissions from municipal incinerators, heating
facilities, and thermal power plants have been the subject of concern.
Whereas previously the emission of dust, smoke, toxic metals, and
noxious gases were of prime concern, the presence of potentially
hazardous organic compounds from these emissions has been recognized
only recently. Lahaniatis et al. (1977) reported the presence of
chlorinated organic compounds (chlorinated aliphatics, benzenes, PCBs,
and pesticides) in fly ash from a municipal incinerator.

Olie et al. (1977) reported the occurrence of PCDDs and PCDFs in
fly ash from three municipal incinerators in the Netherlands. Their
results indicated the presence of up to 17 PCDD peaks, but isomer
identification and quantification was not possible due to the lack of
synthetic standards. Buser & Bosshardt (1978) studied fly ash from a
municipal incinerator and an industrial heating facility, both in
Switzerland. In the former, the level of PCDDs was 0.2 µg/g and of
PCDFs 0.1 µg/g. In the industrial incinerator, the levels were 0.6
µg/g and 0.3 µg/g, respectively.

During the period 1978-1982 a series of papers, reports, and
reviews were published confirming the original findings of Olie et al.
(1977) and Buser & Bosshardt (1978) regarding fly ash. Less data have
been published on the levels of PCDDs and PCDFs in other incineration
by-products, e.g., particulates and flue gas condensate, and in total
flue gas, which are the true emissions (Marklund et al., 1986).

A risk evaluation should be based on the emission levels of PCDD
and PCDF isomers found in isomer-specific analyses using validated
sampling and clean-up methods. However, in many studies non-validated
sampling and analytical methods are used and the results are given in

terms of total levels of tetra-, penta-, hexa-, hepta-, and octaCDDs
and CDFs. The value of such studies is limited, particularly in this

situation where the number of isomers is quite large. More than 30 PCDDs and 60 PCDFs have been found in fly ash samples (Buser et al., 1978b, 1978c).

In March 1986, a working group of experts convened by the World Health Organization Regional Office for Europe reviewed the available data on emissions of PCDDs and PCDFs from municipal solid-waste (MSW) incinerators. It was found that the origin of these compounds was not completely understood, but they appear to result from complex thermal reactions occurring during periods of poor combustion. Because of their high thermal stability, the PCDDs and PCDFs can be destroyed only after adequate residence times at temperatures above 800 °C (WHO/EURO, 1987).

Available data on total emissions of PCDDs and PCDFs from tests on MSW incinerators range between a few and several thousand ng/Nm^3 dry gas at 10% carbon dioxide (CO_2). The working group prepared a table giving a range of estimated isomer specific emissions for those isomers of major concern with respect to MSW incinerators operating under various conditions (Table 17).

The emissions tabulated in column 1 are those which the working group considered to be achievable in the most modern, highly controlled, and carefully operated plants in use at the present time. Such results do not represent what is considered to be achievable by the use of acid gas cleaning equipment; use of such equipment should result in much lower values (probably at least one order of magnitude). The results given in column 1 are not representative of emissions that might be expected from such plants during start-up or during occasional abnormal conditions. Emission levels listed in column 2 were considered by the working group to be indicative of the higher limit of emissions from modern MSW incinerators. These plants might experience such emissions during start-up or during occasional upset conditions. Consequently, the majority of the available concentration data falls between columns 1 and 2. Some of the data reviewed has shown that the figures in column 2 should not be considered an absolute maximum. However, most existing plants, if carefully operated, will have PCDD and PCDF emisions in the range between columns 1 and 2.

The highest values for MSW incinerators (column 3) were obtained by multiplying the values in column 2 by a factor of 5. Column 3 includes emission data that were reported to the working group from all tests and under all circumstances. Generally, these emission levels are associated with irregular or unstable operating conditions, high moisture content of the MSW, low combustion or afterburner

temperatures, less than adequate technologies, etc.

Table 16. Formation of PCDDs and PCDFs by thermal processes

Precursor	Conditions	Products
2,4,5-T salt	Pyrolysis	2,3,7,8-tetraCDD
2,4,5-T (vegetation)	Pyrolysis	No TCDD
	Burning	No TCDD
Cl-phenate	Burning	PCDDs[a] + PCDFs
PCBs	Pyrolysis	PCDFs[b]
PCBz[c]	Pyrolysis	PCDFs + PCDDs[d]
Cl-Diphenyl ethers	Pyrolysis	PCDFs + PCDDs
Cl-Alkanes (Paraffins)	Pyrolysis	PCDFs
PVC	Pyrolysis	PCDDs + PCDFs

[a] = PCDDs formed by dimerization and a non-specific dechlorination.
[b] = other products: hexa- and pentaCBs.
[c] = polychlorinated benzenes.
[d] = other products: PCBs, polychlorinated naphthalenes.

     The working group was aware of both lower and higher emission
levels than those included in Table 17. However, it was felt that the
values included in Table 17 were likely to be representative of
emissions from current facilities (WHO/EURO, 1987).

     Of special importance is the observation that the emission of
1,2,3,7,8-pentaCDD normally exceeds the emission of 2,3,7,8-tetraCDD
by a factor of three to ten.

3.5.3 Incineration of sewage sludge

     Sludge from municipal waste water treatment plants may be
incinerated after being dewatered. The WHO working group (see 3.5.2)
reviewed the available data from municipal sewage sludge (MSS)
incinerators, and found that PCDD and PCDF emissions from this type of
plant were generally lower than emissions from MSW incinerators (see
Table 17, column 4) (WHO, 1986).

3.5.4 Incineration of hospital waste

     Doyle et al. (1985) claimed that the incomplete combustion of

certain hospital waste containing halogenated organics could produce high levels of PCDDs and PCDFs. They found the mean values of total PCDDs to be 69 ng/m^3 and total PCDFs to be 156 ng/m^3. No isomer-specific data seems to be available. Hagenmaier et al. (1986) reported the analyses of stack gas from 10 hospital waste incineration plants. The mean value of 2,3,7,8-tetraCCD emitted was 0.28 ng/m^3, the mean of all TCDDs being 20 ng/m^3. The mean value for total PCDDs was 118 ng/m^3 and for total PCDFs 434 ng/m^3.

Table 17. Estimated range of emissions from municipal solid waste (MSW) and municipal sewage sludge (MSS) incineratorsa

Congeners	Emissions from MSW combustion			Emissions from MSS combustion
	2 with acid cleaning plants	3 Maximum from average operation	4 High emissions	1 Achievable with most modern likely highest gas emissions with no gas cleaning
	(ng/Nm^3, dry, at 10% CO_2)			
2,3,7,8-TetraCDD	0.1	1.5	7.5	0.1
1,2,3,7,8-PentaCDD	0.3	14	70	0.3
1,2,3,4,7,8-HexaCDD				

```
0.2              31                  155                    0.2
          1,2,3,6,7,8-HexaCDD
0.6              56                  280                    0.6
          1,2,3,7,8,9-HexaCDD
0.4              20                  100                    0.4
          2,3,7,8-TetraCDF
0.9              10                   50                    0.9
          1,2,3,7,8-/1,2,3,4,8-PentaCDF
2.3              52                  260                    2.3
          2,3,4,7,8-PentaCDF
2.0              40                  200                    2.0
          1,2,3,4,7,8/1,2,3,4,7,9-HexaCDF
1.1              48                  240                    1.1
          1,2,3,6,7,8-HexaCDF
1.3              40                  200                    1.3
          1,2,3,7,8,9-HexaCDF
0.06             52                  260                    0.06
          2,3,4,6,7,8-HexaCDF
2.0              36                  180                    2.0
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a   From: WHO/EURO (1987).

3.5.5 Incineration of hazardous waste

       Analyses from a test burn of pentachlorophenol waste have been
reported by Rappe et al. (1983c). PCP is a well known precursor to
octaCDD (section 2). Samples of baghouse ash and bottom ash were
analyzed. In the baghouse ash the total level of octaCDD was only 0.2
µg/g. The major constituents were lower chlorinated PCDDs such as
hepta-, hexa-, penta-, and tetraCDDs. The isomeric distribution was
reported to be very similar to a "normal" fly ash. In both cases
2,3,7,8-tetraCDD was a very minor constituent. The level of PCDD in
the bottom ash was 0.31 µg/g. The baghouse ash was also reported to
contain PCDFs at a total level of 2.5 µg/g. For the tetra- and penta-
chlorinated compounds, equal amounts of PCDDs and PCDFs were reported.

       Oberg & Bergstrém (1986) reported on test data from a Swedish
hazardous waste incinerator equipped with a rotary kiln, an
afterburner, and a dry scrubbing unit. Combustion tests were performed
with PCB (Aroclor 1242) as a fluid, and as a contaminant in solid
waste (Aroclor 1016 in capacitors). The results of these tests
indicated no correlation between the amount of PCB incinerated and the

amount of PCDDs and PCDFs found in the emissions.

3.5.6 Metal industry and metal treatment industry

It has been reported by Marklund et al. (1986) that industrial high-temperature processes like copper smelters and electric arc furnaces in steel mills have been identified as sources of environmental contamination by PCDDs and PCDFs. The results are reported in "TCDD equivalents" according to US EPA (1987). The emission from the copper smelter contained 11 ng of TCDD equivalents/Nm^3 dry gas and 10% CO_2, while the dust from the steel mill contained 0.8 ng TCDD equivalents/g dust. Marklund et al. (1986) also considered the emissions from industrial incinerators to be of the same magnitude, or even higher, than the emissions from MSW incinerators.

Southerland et al. (1987) analyzed emissions from various incinerators within Tier 4 of the USA. The highest levels were found in a secondary copper smelter, which contained 170 ng of 2,3,7,8-tetraCDD/Nm^3 and 3% oxygen. This was by far the highest level found within the US EPA National Dioxin Strategy.

3.5.7 Wire reclamation

Hryhorczuk et al. (1981) studied a wire reclamation incinerator in the USA. Using a non-isomer-specific analytical method, they determined total levels of tetraCDDs and tetraCDFs. Two samples were analyzed, one from the furnace and one from the stack. The furnace sample contained 58 ng/kg of total TCDDs and 730 ng/kg of total TCDFs, whereas the stack sample contained 410 ng/kg of total TCDDs and 11 600 ng/kg of total TCDFs.

3.5.8 Traffic

Marklund et al. (1987) reported a study where automobile exhaust emissions were analyzed for PCDDs and PCDFs. Two groups of test cars were utilized: (1) cars equipped with a catalytic converter using unleaded gasoline with no halogenated scavengers; (2) cars with no catalytic converter using leaded gasoline (0.15 g/litre) and a dichloroethane scavenger (0.1 g/litre). Before the test runs, the motor oil was changed in all cars. No PCDDs and PCDFs could be identifed in the cars using the unleaded gasoline, while the average emission from the cars running on leaded gasoline was found to be 30-540 pg/kg of TCDD equivalents. It was assumed that the chlorinated scavenger (dichloroethane) was the precursor of the PCDDs and PCDFs

formed. It was estimated that the total amount of PCDDs and PCDFs from cars in Sweden using leaded gasoline with halogenated scavengers is in the range of 10-100 g TCDD equivalents/year.

3.5.9 Fires and accidents in PCB-filled electrical equipment

In February 1981 a fire in the State Office Building in Binghamton, New York, USA, caused a transformer to rupture, releasing soot throughout the building. The dielectric fluid in the transformer consisted of a mixture of PCB (65%) and chlorinated benzenes (35%). The soot was found to be highly contaminated with PCDFs (total PCDFs > 2000 µg/g). The most toxic isomers (2,3,7,8-tetraCDF; 1,2,3,7,8- and 2,3,4,7,8-pentaCDF; and 1,2,3,4,7,8- and 1,2,3,6,7,8-hexaCDF) were found to be the major constituents within each group of congeners. Levels reported were 12 mg/g of 2,3,7,8-tetra CDF, 670 mg/g of total penta-CDFs, and 965 mg/g of total hexa-CDFs, 46 mg/g of total hepta-CDFs, and 460 mg/g of octa-CDFs (Rappe, 1984; Rappe et al., 1985b). In addition, a series of PCDDs were identified, including the highly toxic 2,3,7,8-tetraCDD, and 1,2,3,7,8-pentaCDD (Rappe et al., 1983a; Buser & Rappe, 1984). It is assumed that the chlorinated benzenes were the dioxin precursors.

Between 1981 and 1985, a series of transformer accidents (7 in all) similar to the one in Binghamton were reported in the USA and Canada (Rappe et al., 1986a). In January 1985, an explosion followed by a fire ruptured a transformer in the basement of a residential complex in Rheims, France. The transformer was filled with PCB (60%) and trichlorobenzene (40%). Total levels of PCDFs were as high as 2570 µg/m^2 before clean-up. Only traces of hepta- and octaCDD were found (Rappe et al., 1985a).

In Europe, between 1981 and 1985, 19 accidents involving indoor capacitor fires and explosions were reported from Scandinavian countries (Rappe et al., 1986a). All capacitors were mineral-oil filled, and contamination of the sites averaged 1-5 µg total PCDFs/m^2.

3.5.10 Pulp and paper industry

Large amounts of chlorine or chlorine compounds are used in the pulp and paper industry for the bleaching of the pulp. Three black liquor boilers from the craft paper process were included in the US EPA study of combustion sources. No 2,3,7,8-tetraCDD was found in these emissions, but low levels of other PCDDs and PCDFs were found in one of the three incinerators and a yearly emission of 0.25 g was calculated (Southerland et al., 1987).

Rappe et al. (1987) recently identified both 2,3,7,8-tetraCDD (170 ng/kg) and 2,3,7,8-tetraCDF (890 ng/kg) in a sample taken in a sedimentation lagoon at a Swedish paper mill. A series of other PCDDs and PCDFs was also identified but at lower levels. The isomeric pattern in this sample differed markedly from other sediment samples, indicating pulping processes to be a source of environmental pollution by 2,3,7,8-tetraCDD and 2,3,7,8-tetraCDF (Rappe et al., 1987).

3.5.11   Incineration of coal, peat, and wood

The emissions of PCDDs and PCDFs from coal-fired power plants (Kimble & Gross 1980), wood stoves (Clement et al., 1985), and peat burning (Marklund et al., 1986) seem to be very low when calculated per m^3. However, the very high flow rates and the large number of units could make a significant total contribution. The occurrence of pentaCDDs and all PCDFs was not discussed in this report.

3.5.12   Inorganic chlorine precursors

It is well known that certain organochlorine compounds are efficient precursors to PCDDs and PCDFs during pyrolysis. However, it was proposed by scientists from Dow Chemical Company that PCDDs, and especially 2,3,7,8-tetraCDD, are ubiquitous and formed as trace level by-products of any normal combustion (Bumb et al., 1980). Consequently, dioxins should have been present in the environment since the advent of fire. This suggests that inorganic chloride can serve as a useful precursor to the formation of PCDDs and PCDFs. A recent survey of PCDD levels, in particular from residential wood combustion units, has been quoted in support of the above. The survey showed PCDD levels in the ng/kg range (see also section 3.5.11). However, this hypothesis has been criticized. One of the main arguments against such a hypothesis is that 2,3,7,8-tetraCDD does not appear to be formed in coal-fired power plants (Kimble & Gross, 1980; Junk & Richard, 1981). Another argument is that the Dow studies lack data on levels of dioxin precursors in the material being burned, including the air in the flames (Rappe, 1984).

The analyses of historical samples gives additional support to the theory that organochlorine compounds are more important as precursors than inorganic chloride. When Czuczwa & Hites (1985) analyzed sediment core samples from Lake Huron, N. America, the first indication of PCDDs and PCDFs was found in sediments from 1940. There was also a good correlation between the trend in the levels of PCDDs and PCDFs in these sediments and the trend in the production of chlorinated aromatic compounds (section 5.4).

Schecter et al. (1986a) were unable to detect PCDDs and PCDFs in human liver and lung tissue from two female Eskimos frozen over one hundred years ago (see also section 4.4.4).

3.5.13 Photochemical processes

Sundström et al. (1979) studied the formation of 2,3,7,8-tetraCDD in six re-forestation areas that were sprayed with 2,4,5-T esters. Leaf samples from the areas were analyzed for 2,4,5-T esters and TCDD. TCDD was found in one leaf sample only, at levels lower than expected from the level of dioxin contamination of the herbicide formulations used.

The photochemical formation of PCDDs and PCDFs has also been studied in laboratory experiments.

The photochemical dimerization of chlorophenols to PCDDs was studied by Crosby & Wong (1976). The only PCDD formed in this study was the octaCDD. Other PCDDs can be formed by photochemical cyclization of chlorinated o-phenoxyphenols, also called pre-dioxins (Nilsson et al., 1974). These pre-dioxins are very common impurities (1-5%) in commercial chlorophenols (Nilsson et al., 1978), but the cyclization is only a minor reaction pathway; the main reaction being the dechlorination of the pre-dioxin.

Akermark (1978) studied the formation of 2,3,7,8-tetraCDD from the appropriate pre-dioxins. He could identify the product, but claimed the reaction to be very inefficient.

Another photochemical process of potential environmental importance is dechlorination of the higher chlorinated PCDDs and PCDFs, e.g., octaCDD and octaCDF. The products formed by photolysis of octaCDD in organic solvent have now been identified (Buser & Rappe, 1978). By comparison with authentic standards, it was found that the main tetrachloro isomer was the 1,4,6,9-tetraCDD; the major pentachloro compound was expected to be the 1,2,4,6,9-isomer, and the main hexa- and heptachloro compounds were the 1,2,4,6,7,9- (or 1,2,4,6,8,9-) and the 1,2,3,4,6,7,9-isomers, respectively. The reaction scheme deduced from this data indicates that the chlorine

atoms are removed preferentially from the lateral positions on the carbon rings. Consequently, the most toxic PCDD isomers, such as 2,3,7,8-tetraCDD, are not likely to be formed from the photolysis of the higher PCDDs in solution.

Crosby et al. (1973) studied the photolysis of a series of PCBs dispersed in water. For two isomers, the 2,5-dichloro- and 2,2',5,5'-tetrachlorobiphenyls, small amounts (0.2%) of 2-mono-CDF could be found among the products (for photo-chemical transformations, see section 4.2.1).

3.6 Comparison of Isomeric Pattern and Congener Profiles From Various Sources

There is a pronounced difference between technical products and incineration emissions in both isomeric patterns and congener profiles of PCDDs and PCDFs. In technical products the number of isomers present is limited, whereas in incineration emissions most isomers seem to be present. Rappe (1987) has pointed out the large similarity qualitatively in isomeric patterns between different incineration sources.

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATIONS

4.1 Environmental Transport

4.1.1 Air

The PCDDs and PCDFs are believed to be transported in the atmosphere. The transport of these compounds from stacks and other stationary point sources, as well as from waste disposal sites and other area sources, can be predicted from dispersion modelling (SAI, 1980). In the case of the accidental release of a toxic cloud containing 2,3,7,8-tetraCDD at Seveso, Italy, Cavallaro et al. (1982) determined the transport pattern and the ground deposition. They determined that the deposition of 2,3,7-8-tetraCDD from air to soil should follow an exponential decay pattern in the Gaussian-distribution along the cross-section of the downwind direction. Thibodeaux (1983) studied the air transport of 2,3,7,8-tetraCDD at a herbicide production facility in Jacksonville, Arkansas, USA.

The dispersion modelling has limitations. If possible, the modelling calculations should be combined with true air measurements.

4.1.2 Water

The solubility of 2,3,7,8-tetraCDD in water has been extensively studied (see section 2), but much less data are available for the other PCDDs and PCDFs. However, data from microbiological experiments indicate that 2,3,7,8-tetraCDD is highly adsorbed to sediments and biota. Matsumura et al. (1983) suggested that more than 90% of the

2,3,7,8-tetraCDD in an aquatic medium could be present in the adsorbed
state. Rappe et al. (1985c) studied a suspension of soot/dust in the
wash water from a PCB fire. The suspension contained 100 ng/ml of
various PCDFs, but when the soot was settled the water contained no
detectable levels of PCDFs (detection level: 0.1 ng/ml of each
isomer). Most of the PCDDs and PCDFs, if present in waterways, should
be in the sediments or attached to suspended particles.

Thibodeaux (1983) has calculated the amount of 2,3,7,8-tetraCDD
transported by a creek within the contaminated herbicide factory in
Jacksonville, Arkansas, USA. The value was 0.89 g/year as an average
rate, and a maximum of 2.1 g/year.

4.1.3 Soil and sediments

The mobility of 2,3,7,8-tetraCDD and of a dichlorodioxin in soils
has been studied by Helling et al. (1973). Both were found to be
immobile in all soils and, therefore, would not be leached out by
rainfall or irrigation, though lateral transport during surface
erosion of the soil could occur.

The US Air Force conducted studies in an area of north-west
Florida that had been heavily sprayed with the herbicide Agent Orange
between 1962 and 1964 (Young et al., 1975). This herbicide mixture was
contaminated with TCDD (section 3.2). A 7.8-ha test grid received a
total of 40 metric tons of 2,4,5-T between 1962 and 1964. When 15-cm
soil core samples were taken in 1974, they showed TCDD concentrations
ranging from 10 to 710 ng/kg. This study illustrates that significant
levels of TCDD residues remained 10 years after the last herbicide
application. Similar TCDD concentrations were obtained from areas that
had been sprayed between 1962 and 1969 (Bartelson et al., 1975).

In another study Young (1983) measured the concentration of
2,3,7,8-tetraCDD in a soil profile. The samples were collected in 1974
and the data suggested that most of the 2,3,7,8-tetraCDD would be
found in the top 15 cm of the soil profile (Table 18).

The probable media and modes of transport of PCDDs from soils are
the following: (1) to air via contaminated airborne dust particles;
(2) to surface water via eroded soil transported by water; (3) to
groundwater via leaching; (4) to air via volatilization. Movement of
particulate matter containing adsorbed PCDDs and PCDFs has been
considered to be a much more important transport mechanism than
leaching and volatilization because of the low water solubility and
volatility of these compounds (Josephson, 1983). However, the
monitoring of Seveso soil one year after the accident showed that the

highest 2,3,7,8-tetraCDD levels were not present in the topmost soil
layer (0.5 cm), but very often in the second (0.5-1.0 cm) or third
(1.0-1.5 cm) layers. This disappearance of at least a part of the
2,3,7,8-tetraCDD from the topmost soil layer was speculated to be due
to volatilization or vertical movement through the soil (DiDomenico et
al., 1980). Therefore, it appears that volatilization from soil and
leaching to groundwater can be responsible for the transport of PCDDs
and PCDFs from soils under certain conditions, namely, heavy rainfall
on sandy soils. Studies by Young (1983) indicate that the half-life
for 2,3,7,8-tetraCDD in soil is 10-12 years.

Thibodeaux (1983) has calculated the vaporization of
2,3,7,8-tetraCDD from a herbicide plant in Jacksonville, Arkansas,
USA. The vaporization can take place from soil surfaces, from landfill
cells, and from the surface of a pond. In Table 19 a summary of yearly
emission rates from these sources is presented.

It was found that vaporization from the soil surface in the
highly contaminated blow out area was the major contributing source of
emissions from this plant.

Table 18. Concentration of 2,3,7,8-tetraCDD in a soil profile[a], [b]

Depth (cm)	2,3,7,8-tetraCDD (ng/kg)
0 - 2.5	150
2.5 - 5.0	160
5.0 - 10	700
10 - 15	44
15 - 90	ND[c]

[a]   From: Young (1983).

[b]   The area received 1,069 kg/ha of 2,4,5-T Agent Orange during
       1962-1964. The soil samples were collected and analyzed in 1974.

[c]   None detected (minimum detection limit: 10 ng/kg).

Table 19. Surface source areas and emission rates of 2,3,7,8-tetraCDD[a]

Source	Area (m^2)	Emission rate (g/year)

Blow-out area, volatilization	753	120-1200
Blow-out area, entrainment	753	28-37
Rocky Branch Creek, dissolved		0.89-2.1
Reasor-Hill dump	1129	0.1-1.0
Rocky Branch Creek, sediment		0.094-0.22
Cooling water pond	15050	0.015-0.016
Total		150-1240

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a From: Thibodeaux (1983).

Freeman et al. (1986) have developed a model to describe the vaporization and diffusion through a column of soil of low volatility organic chemicals like PCDDs and PCDFs. This model has been used to make predictions on the transport of 2,3,7,8-tetraCDD at a site in Times Beach, Missouri, USA. The model predicted that the 1983 levels in this soil would be only 10% of the original loading. The model also predicted that 57% of the initial amount of 2,3,7,8-tetraCDD was vaporized through the soil column to the surface in the first year after the spraying and that most transport of the vapour occurred during the summer months. The same results were also obtained in studies reported by Facchetti et al. (1986) and Palausky et al. (1986).

4.2 Environmental Transformation

4.2.1 Abiotic transformation

Like other PCDDs and PCDFs, 2,3,7,8-tetraCDD is chemically quite stable, and is not likely to be degraded at a significant rate by hydrolytic reactions under environmental conditions. Under these conditions, TCDD seems also to be rather stable to photochemical degradation (Crosby et al., 1971). The half-life of TCDD of about 10-12 years, as found by Young et al. (1983) for soil, is in agreement with this observation.

However, three reports on rapid photochemical degradation of 2,3,7,8-tetraCDD under experimental conditions make the situation more complicated. In a methanol solution, TCDD is fairly easily degraded by photolysis in the laboratory (Crosby et al., 1971). Other studies using 2,4,5-T ester formulations with known amounts of TCDD and exposed to natural sunlight on leaves, soil, or glass plates showed that most of the TCDD was lost during a single day (Crosby & Wong,

1977). In these two studies, a "hydrogen donor", such as methanol or
2,4,5-T ester, enhanced the photochemical dechlorination (Akermark,
1978); they do not therefore truly reflect environmental conditions,
where the 2,4,5-T ester would be rapidly hydrolyzed on the surface of
the leaves. At Seveso the TCDD was released together with salts of
2,4,5-trichlorophenol, ethylene glycol, and inorganic constituents
(Rappe, 1978b). Like water, none of these is a potent hydrogen donor.

     According to Bertoni et al. (1978), the addition of a solution of
ethyl oleate in xylene enhances the breakdown of TCDD in soil by UV
light. Similarly, a cationic surfactant, 1-hexadecylpyridinium
chloride, was also reported to enhance photodecomposition (Botre et
al., 1978).

     Another experiment, which might be a good model for the
degradation of TCDD bound to dust particles in the air, has shown that
TCDD adsorbed on silica gel undergoes rapid photo-chemical degradation
(Gebefugi et al., 1977).

     In order to explain the longer half-life of 2,3,7,8-tetraCDD in
a model laboratory ecosystem than in an outdoor pond, Matsumura et al.
(1983) speculated that photolysis was the most likely cause. In the
outdoor environment, algae-mediated photosensitization of
2,3,7,8-tetra-CDD may have caused some photodecomposition of this
compound.

     An increase in chlorine substitution is expected to decrease the
rate of photodegradation. For example, Crosby et al. (1971) showed
that although complete decompostion of 2,3,7,8-tetraCDD in methanol
occurred in 24 h under UV irradiation, > 80% octaCDD in methanol
remained unreacted during the same period under similar irradiation
conditions.

     Although the degree of photolysis may be related to the extent of
chlorination, different chlorine substitution patterns also play a
critical part. In higher chlorinated PCDDs, there appears to be
preferential loss of chlorine from the 2,3,7, and 8 positions (Buser
& Rappe, 1978). Thus, PCDDs with chlorine substitutions in positions
2,3,7, and 8 are likely to be photochemically degraded faster than
compounds not having these positions substituted. For example, the
photolysis half-life of 1,2,3,7,8-pentaCDD has been estimated to be
7.8 h in [n]-hexadecane solution under sunlamp irradiation (Nestrick
et al., 1980). Similarly, the photolytic half-lives of
1,2,3,7,8-pentaCDD, 1,2,3,6,7,9-, and 1,2,4,6,7,9-hexaCDD in hexane
solutions under sunlight irradiation have been determined to be 5.4,
17, and 47 hours, respectively (Dobles & Grant, 1979). Nestrick et al.

(1980) reported a half-life value of 6.8 h for 1,2,3,6,7,8-hexaCDD in n-hexadecane under sunlamp irradiation. The primary intermediates of the photo-degradation of higher chlorinated PCDDs are probably lower chlorinated dioxins (Buser & Rappe, 1978), but the pathways of degradation are not known with certainty (National Research Council of Canada, 1981).

From these discussions of the photolysis of PCDDs in the presence of organic hydrogen-donating substrates, it is difficult to predict the photolytic fate of these compounds in natural aquatic media, where hydrogen donors may or may not be available. The situation is complicated further by the fact that a predominant amount of PCDDs in surface water may be adsorbed or suspended on particles and sediments, rather than in solution. Moreover, since the penetration of UV light into natural water may be very limited, photolytic degradation of PCDDs in water is not likely to be of environmental importance.

Hutzinger et al. (1973) have studied the photochemical degradation of 2,8-diCDF and octaCDF. They found that a reductive dechlorination takes place, especially in methanolic solution. The reaction was much slower when a thin film was exposed to sunlight.

Thermally, 2,3,7,8-tetraCDD is quite stable, rapid decomposition occurring only at temperatures above 750 °C (Stehl et al., 1973).

4.2.2 Biotransformation and biodegradation

The 2,3,7,8-tetraCDD isomer is very resistant to biodegradation. Only 5 of about 100 microbial strains with the ability to degrade persistent pesticides were able to degrade 2,3,7,8-tetraCDD (Matsumura & Benezet, 1973). Ward & Matsumura (1978) studied the biodegradation of [14]C-labelled 2,3,7,8-tetraCDD in lake waters and sediments from Wisconsin, USA, and observed a half-life of 2,3,7,8-tetraCDD in lake waters containing sediment of 550-590 days. In lake water alone, about 70% of the 2,3,7,8-tetraCDD remained after 589 days. Using an outdoor pond as a model aquatic ecosystem, and dosing it with [14]C-labelled 2,3,7,8-tetraCDD, Matsumura et al. (1983) estimated the half-life of 2,3,7,8-tetraCDD to be approximately 1 year. Although biodegradation

may have been responsible for part of the degradation, it is almost impossible to estimate the biodegradation half-life of 2,3,7,8-tetraCDD in aquatic systems from this experiment.

Philippi et al. (1982) detected a polar metabolite of 2,3,7,8-tetraCDD in several microbiological cultures after long-term incubation. They reported chromatographic and MS data that supported

the conclusion that the metabolite was 1-hydroxy-2,3,7,8-tetraCDD, although a synthetic standard compound was not available.

Tulp & Hutzinger (1978) reported that in rats, dibenzo-p-dioxin, 1-monoCDD, 2-monoCDD, 2,3-diCDD, 2,7-diCDD, 1,2,4-triCDD, and 1,2,3,4-tetraCDD are metabolized to mono- and di-hydroxy derivatives. In the case of dibenzo-p-dioxin and both of the two monochloro isomers, sulfur-containing metabolites were also excreted. Primary hydroxylation exclusively took place at the 2, 3, 7, or 8 positions in the molecule. In these studies, no metabolites resulting from fission of the C-O bonds (ortho, ortho'-dihydroxychlorodiphenyl ethers, chloro-catechols), or hydroxylated derivatives thereof, were detected. No metabolites were found from octaCDD.

4.3 Bioaccumulation

The bioaccumulation of 2,3,7,8-tetraCDD has been investigated in several studies, using several aquatic species and different model ecosystems. In the experiments in which [14]C-TCDD was introduced into the model ecosystem in the form of residues on sand, particularly high values were found in the mosquito (Aedes egypti) larvae, the level exceeding that found in water by more than 9000 times. Under similar conditions, the level in brine shrimp (Artemia salina) was 1570 times higher than that found in water (Matsumura & Benezet, 1973). In the second study (Isensee & Jones, 1975; Isensee, 1978), [14]C-TCDD was absorbed, at a broad range of levels, into soil and placed at the bottom of an aquarium. Five species of organisms were added (though not simultaneously) 1-30 days after flooding, and exposed for 3-32 days. The correlation between the TCDD level in the water and in the organisms of each species was highly significant (correlation coefficient of 0.94 or higher).

Bioaccumulation factors for 2,3,7,8-tetraCDD are given in Table 20 (US EPA, 1985).

4.4 Levels in Biota

4.4.1 Vegetation

When [14]C-labelled 2,3,7,8-tetraCDD was added to soil, both oats and soya beans accumulated small quantities of TCDD, at all stages of growth. TCDD was also detected in control plants housed with the experimental plants after treatment (Isensee & Jones, 1971). A maximum of 0.15% of the TCDD present in the soil was translocated to the

aerial portion of the oats and the soya beans, but neither the grain

nor the beans harvested at maturity showed any detectable level of ^{14}C-labelled TCDD. When TCDD was applied to the central leaflet of 3-week-old soya bean plants and 12-day-old oat plants, very little TCDD was lost from the soya bean leaves in 21 days, but there was a gradual loss (38% in 21 days) from the oat leaves.

Analyses of vegetation from Seveso, Italy, after the industrial accident, gave values of up to 50 mg TCDD/kg (Firestone, 1978). In the following years, when there was no direct contact of the newly grown vegetation with the aerosol cloud, the levels of dioxin in plants decreased by several orders of magnitude (Wipf & Schmid, 1983). In 1977 (one year after the accident in Seveso), no traces of TCDD were found in the flesh of apples, pears, and peaches, or in corn cobs or kernels, grown near the factory (the detection limit for the analyses was 1 ng/kg). At the same time about 100 ng TCDD/kg was detected in the fruit peels. This strongly suggests that the contamination was due to dust and not from plant uptake. The TCDD level in the soil was found to be in the order of 10 ng/g, which corresponds to about 1000 µg/m^2 (Wipf et al., 1982).

Facchetti et al. (1986) studied plants grown in soil spiked with 2,3,7,8-tetraCDD in the range 1-752 ng TCDD/kg. At the end of cultivation, root samples were collected, carefully washed, and analyzed. The levels of TCDD in the roots were found to be higher than the levels found in the soil in which the plants were grown. On the parts above ground, Facchetti et al. (1986) could not find any significant increase in the levels of TCDD. However, the TCDD concentration was found to vary with the location, being higher if the plants were grown in the vicinity of other pots containing contaminated soil. The conclusion was drawn that evaporation is the predominant process for the contamination of the aerial parts of plants. However, studies by Sacchi et al. (1986) indicated that maize and bean plants grown in soil contaminated by ^3H-2,3,7,8-tetraCDD accumulated radioactivity in the aerial parts progressively with time and with soil contamination (Sacci et al., 1986). It was suggested that the distribution of the TCDD into the leaves occurred via the transpiration stream.

Very few analyses of sprayed vegetation have been reported. A rough estimate of 20-1000 ng/kg for 2,3,7,8-tetraCDD contamination can be made on the basis of the level of 2,4,5-T found in newly sprayed vegetation and the level of 2,3,7,8-tetraCDD in the spray formulation used. Higher values could be obtained for Agent Orange. Sundström et al. (1979) reported data in agreement with this estimate. However, the analytical technique used in their study was not isomer specific. Vegetation was sprayed with 2,4,5-T ester contaminated by only 0.06 mg

2,3,7,8-tetraCDD/g. A sample of leaves collected 42-45 days after the spraying was found to have 170 ng TCDD/kg, somewhat lower than the expected value 600 ng TCDD/kg, indicating a slow photochemical breakdown.

Table 20. Measured bioaccumulation factor for 2,3,7,8-TCDD in freshwater aquatic organisms[a]

Species	Tissue	Duration (days)	Bioconcentration factor	Reference
Alga (Oedogonium cardiacum)		33	3094[b]	Isensee (1978)
Alga (Oedogonium cardiacum)		32	2075[c]	Isensee (1978) Yockim et al. (1978)
Snail (Physa sp.)	whole body	33	5471[b]	Isensee (1978)
Snail (Physa sp.)	whole body	32	3095[c] 3731	Isensee (1978) Yockim et al. (1978)
Cladoceran (Daphnia magna)	whole body	32	3895[b]	Isensee (1978)
Cladoceran (Daphnia magna)	whole body	30	7070[c] 7125	Isensee (1978) Yockim et al. (1978)

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         Catfish                       whole body
28             4875                          Yockim et al. (1978)
         (Italurus punctatus)


         Mosquitofish                  whole body
14             4850c                         Isensee (1978)
         (Gambusia

affinis)
4875                            Yockim et al. (1978)
```

a   From: US EPA (1985).

b   Arithmetic mean of several values reported.

c   Tissue concentrations at equilibrium.

Table 21. Levels of TCDDs in fish and shellfish[a]

Sample number	Tissue type[b]	Concentration of 2,3,7,8-TCDD (ng/kg)[c]
1	Fish (edible flesh)	480
2	Catfish	40
3	Buffalo fish	ND(13)
4	Fish (predator)	230
5	Fish (bottom feeder)	77
6	Catfish	50
7	Buffalo fish	ND(7)
8	Catfish	ND(7)

a   From: Mitchum et al. (1980).

b   All samples were obtained from the Arkansas River, USA, or a tributary, the Bayou Meto.

c   These are averages of samples that had detectable levels of TCDD.

ND = none detected; the number in parenthesis is the measured detection limited for that sample.

4.4.2 Aquatic organisms

     Fish and shellfish taken from areas in South Viet Nam that were
heavily exposed to Agent Orange during military defoliation operations
in the 1960s have been reported to contain 18-810 ng TCDD/kg (Baughman
& Meselson, 1973). The analytical technique of direct-inlet high
resolution MS used in this study is not considered isomer specific and
did not include any GC separation at all.

     In two streams associated with the US Air Force test area in
north-west Florida (section 4.1.3), which had been heavily sprayed
with Agent Orange between 1962 and 1964, the silt contained, 10 years
later, 10 and 35 ng TCDD/kg where eroded soil entered the water.
Concentrations of 12 ng TCDD/kg were found in two species of fish from
this stream, the sailfin shiner (Notropis hypselopterus) and the
mosquito fish (Gambusia affinis). The spotted sunfish (Lepomis
punctatus) contained 4 ng TCDD/kg in skin and muscle, 18 ng/kg in the
gonads, and 85 ng/kg in the gut (Young et al., 1976).

     Table 22. Analytical results for 2,3,7,8-tetraCDD residues in fish from
Saginaw Bay Region, Michigan, USA[a]

Species	Number of samples[b]	Number of positive samples	TCDD detected (ng/kg)[c] low	high	mean
Channel catfish	8	8	28	695	157 (13)
Carp	14	10	20	153	55 (7)
Yellow perch	6	3	10	20	13 (5)
Smallmouth bass	2	2	7	8	8 (6)
Sucker	4	3	4	21	10 (4)
Lake trout	2	0	0	0	0 (5)

a   From: Harless et al. 1982).

b   Mean % recovery for 2.5-10 ng $^{37}Cl4$-TCDD added to 5 or 10
        g of tissue prior to sample preparation was between 78 and
        100%.

c   Corrected for losses in efficiency of sample preparation for
        particular species. The numbers in parenthesis indicate the
        limit of detection for TCDD.

     The levels of TCDD in fish from the Atlantic or from ponds in the
USA in areas sprayed with 2,4,5-T were below the detection levels (1-2
ng/kg) (Baughman, 1974; Shadoff et al., 1977).

     Mitchum et al. (1980) reported levels of 400 ng
2,3,7,8-tetraCDD/kg in fish samples collected in Bayou Meto/Arkansas
River, USA, a waterway associated with industrial plants for the
production of 2,4,5-T (Thibodeux, 1983) (see Table 21).

     Levels ranging from 4-695 ng TCDD/kg were found in the edible
portion of channel catfish, carp, yellow perch, small-mouth bass, and
suckers from Saginaw Bay, Michigan, USA, near facilities used for the
production of 2,4,5-T herbicides. The highest concentrations were
detected in bottom-feeding catfish and carp, while the lowest
concentrations were detected in bass, perch, and suckers (see Table
22) (Harless et al., 1982).

     Rappe et al. (1981) identified a series of tetra- to octaCDFs in
fat samples of a snapping turtle from the Hudson River and of gray
seal from the Baltic Sea. The total levels of PCDFs in these samples
were 3 ng/g and 40 ng/kg, respectively. In both samples the major
PCDFs consisted of the most toxic isomers (2,3,7,8-tetra-;
2,3,4,7,8-penta-; and 1,2,3,4,7,8- and 1,2,3,6,7,8-hexaCDFs).

     Norstrom et al. (1982) have analyzed pooled samples of herring
gull eggs collected in 1982 from various parts of the Great Lakes, N.
America. In all samples, 2,3,7,8-tetraCDD was found in levels ranging
from 9 to 90 ng/kg. The identity of the 2,3,7,8-isomer was confirmed
by retention times on three capillary columns. In another study
Stalling et al. (1983) were not able to detect measurable levels of
tetraCDDs and other PCDDs in fish samples from Lake Superior,
N.America (the detection level was 2-5 pg/g). The difference could be
explained by the migration of the herring gulls during the winter. On
the other hand, a series of PCDFs could be identified in the Lake
Superior fish samples, indicating more widespread background levels

for the PCDFs than for the PCDDs. Stalling et al. (1983) found the
total levels of PCDFs in fish samples from Lakes Michigan, Huron, and
Ontario, N. America, to be 12-290 ng/kg. The toxic 2,3,7,8-substituted
PCDDs and PCDFs were present in all samples, the highest levels being
found in samples from Lake Huron, Lake Ontario, and the Tittabawasee
River, which flows into Saginaw Bay. The residue pattern found in the
fish and locally high levels suggest a strong influence by local point
source discharges (Stalling et al., 1983). The data of O'Keefe et al.
(1983) are also in agreement with this theory.

Norstrom et al. (1986) have studied the long-term trends of
2,3,7,8-substituted PCDDs and PCDFs in herring gull eggs in the Great
Lakes. The levels of 2,3,7,8-tetraCDD were found to decline
exponentially in Lake Ontario, with a half-life of 3-4 years, from a
high of 2000-5000 ng/kg in the early 1970s to a level of 80-100 ng/kg
in 1984/1985. The levels of TCDD in Lake Michigan were 249 ng/kg in
1971, 70 ng/kg in 1972, and 10-20 ng/kg in 1984/1985. These levels
have not changed significantly since 1979. This suggests that an
equilibrium between input and removal mechanisms has been established
in this water system for most PCDDs and PCDFs. The same trend is
reported for various fish species in Lake Ontario (Ontario, 1986).

Ryan et al. (1983a) analyzed a series of commercial and sport
fish from the Great Lakes and from the Pacific coast of Canada for
2,3,7,8-tetraCDD (Table 23). The highest levels were found in Lake
Huron and Lake Ontario. In a preliminary study they also reported
finding levels of 2,3,7,8-tetraCDFs and other unidentified tetraCDFs
of 3-200 ng/kg of fish.

The Baltic Sea is an area of interest because this region is
without any known point sources of dioxins. Rappe et al. (1987)
reported on the analyses of two samples of homing salmon and two
samples of pooled herring; one herring sample from the Baltic Sea
(Karlskrona) and the other from the northern part of the Gulf of
Bothnia (Lulea) (Table 24). As expected, the levels in the salmon
muscle were much higher than the levels found in the herrings, but,
unexpectedly, the levels in the herring sample from the Gulf of
Bothnia (Lulea) were somewhat higher than levels found in the sample
from the Baltic Sea (Karlskrona).

An interesting observation is that in the majority of the aquatic
samples only the 2,3,7,8-substituted PCDD and PCDF congeners were
found. However, crustaceans seemed to be an exception from this
general trend. Norström et al. (1988) reported that crab
hepatopancreas from the Canadian Pacific Coast contain other
congeners, e.g. 1,2,4,7,8-pentaCDD and

1,2,3,6,7,9-/1,2,3,6,8,9-hexaCDD. Rappe et al. (1987) collected and analyzed crab hepatopancreas from three different locations along the west coast of Sweden. The crab samples from the locations Grebbestad and Idefjord should represent background levels, while Väröfjord has a potential point source of dioxins from a pulp mill using chlorine for bleaching. The results are given in Table 25.

Low background levels of series PCDDs and PCDFs were found in all samples. In addition, the sample from the Väröfjord also contained much higher levels of some congeners, especially 2,3,7,8-tetraCDF and 2,3,7,8-tetraCDD. This is another indication that pulp bleaching could be a potential source of 2,3,7,8-tetraCDD and 2,3,7,8-tetraCDF (see section 3.5.10).

4.4.3 Terrestrial animals

In a heavily sprayed test area in north-west Florida (Young et al., 1976), a total of 106 adult and 67 fetuses of beach mice (Peromysous polionotus) were collected in 1973 and 1974 and examined (method not specified). Livers from the beach mice contained from 540-1300 ng TCDD/kg and the pelts 130-140 ng/kg. The visceral mass of race runners (Cnemidophorus sexlineatus) which were caught in that area contained 360 ng TCDD/kg and the trunk of the reptiles contained 370 ng/kg.

At the time of the accident in Seveso, Italy, more than 81 000 animals were inhabiting the contaminated zones. Most were rabbits (25 000), poultry, and other small animals (55 500), with 349 cattle, 233 pigs, 49 horses, 21 sheep, and 49 goats also in the zones. Many of these animals died and others were killed. A large number of these animals were analyzed for 2,3,7,8-tetraCDD by a method with a detection level of 250 ng/kg (Pocchiari et al., 1983). The results are summarized in Tables 26 and 27.

Table 23. Levels of 2,3,7,8-tetraCDD and PCB in Great Lakes Canadian sport fish (1980) and smelt (1979)[a]

Species	Origin	TCDD (ng/kg)	PCB (µg/g)
Lake trout[b]	Lake Ontario	58	7.28
	Lake Huron	37	5.03
Rainbow trout[b]	Lake Ontario	33	1.77

Coho salmon	Lake Ontario	28[c]	7.39
	Pacific Coast	ND[d] (4)	0.03
Smelt	Lake Ontario	11	
		16	
		11	
	Lake Erie	ND[d] (2)	

[a]   From: Ryan et al. (1983a).

[b]   Whole fish.

[c]   Also contained 36 ng hexaCDD/kg (three isomers) and 93 ng
      octaCDD/kg.

[d]   ND = not detected at bracketed detection limit.

Table 24. Levels of PCDDs and PCDFs in fish samples from the Baltic Sea
(pg/g) [a],[b]

	Salmon Ume River 1985	Salmon Ume River 1985	Herring Karlskrona 1983	Herring Lulea 1983
2,3,7,8-TetraCDF	29	12	5.5	3.0
2,3,7,8-TetraCDD	1.9	1.3	< 0.3	< 0.6
1,2,3,7,8-/1,2,3,4,8-PentaCDF	6.9	3.3	1.4	0.9
2,3,4,7,8-PentaCDF	49.0	23.0	6.8	8.8
1,2,3,7,8-PentaCDD	8.8	4.3	1.1	4.7
1,2,3,4,7,8-/1,2,3,4,7,9-HexaCDF	1.1	0.7	0.4	0.3
1,2,3,6,7,8-HexaCDF	1.3	0.8	0.4	0.3
1,2,3,7,8,9-HexaCDF	ND	ND	0.4	0.2
2,3,4,6,7,8-HexaCDF	1.1	0.6	0.4	0.2
1,2,3,4,7,8-HexaCDD	ND	0.4	0.2	ND
1,2,3,6,7,8-HexaCDD	4.6	2.3	ND	8.1
1,2,3,7,8,9-HexaCDD	ND	ND	ND	ND
Total HeptaCDFs	ND	2.7	0.8	ND
Total HeptaCDDs	ND	ND	ND	ND
OctaCDF	ND	1.0	ND	ND
OctaCDD	ND	ND	ND	ND

Table 24.(cont'd)   Levels of 2,3,7,8-tetraCDD and PCB in Great
Lakes Canadian sport fish (1980) and smelt (1979)[a]

a   From: Rappe et al. (1987).

b   ND indicates a level < 0.1 pg/g.

Harless et al. (1983) reported a study in which 2,4,5-T containing less than 0.1 mg of 2,3,7,8-tetraCDD/kg was applied at a rate of 3.4 kg/ha to approximately 3 ha of an enclosed plot (4.5 ha). Twelve deer were placed in the enclosure prior to the application of 2,4,5-T. One deer died two days later of unknown causes. The remaining deer were sacrificed prior to, and at specific intervals during, the course of the 30-day study. The analytical results are summarized in Table 28.

In another study (Hryhorczuk et al., 1981), samples from a horse grazing close to a wire reclamation incinerator were analyzed and found to contain unspecified tetraCDFs (165 ng/kg in the fat, 57 ng/kg in the liver) and unspecified tetraCDDs (45 ng/kg in the fat and less than 6 ng/kg in the liver) (compare section 3.5.7).

In order to identify PCDD and PCDF levels in the general terrestrial background, Nygren et al. (1986) analyzed bovine samples - fat, liver, and milk - and identified the same 2,3,7,8-substituted PCDDs and PCDFs as were found in the aquatic samples. However, the levels were lower and normally close to the detection limit.

4.4.4 Human data

Occupational exposure to 2,3,7,8-tetraCDD can occur during the production of 2,4,5-trichlorophenol and the subsequent production and use of 2,4,5-T acid and esters. The first commercial production of 2,4,5-T in the United States was in 1944, and the use of 2,4,5-T herbicides increased in the 1940s and 1950s. However, the problem of dioxin contamination in 2,4,5-T was not recognized until 1957 (Kimmig & Schulz, 1957a, b).

During the normal production of 2,4,5-T, the heaviest exposure to TCDD is during purification steps. The residues are far more contaminated than the purified products. Only limited information is available on the levels of TCDD contamination of products prepared prior to the 1970s, and absolutely no information is available on the dioxin levels in the corresponding residues. Consequently it is a difficult task to estimate the levels of occupational and general population exposures during the period prior to 1970.

Table 25. Levels of PCDDs and PCDFs in samples of crab hepatopancreas from the west coast of Sweden[a]

	Crab Idefjorden (pg/g)	Hepatopancreas Grebbestad (pg/g)	Väröfjord (pg/g)
2,3,7,8-tetraCDF	31	47	590
Total tetraCDFs	90	114	800
2,3,7,8-tetraCDD	17	17	170
Total tetraCDDs	17	17	170
1,2,3,7,8-pentaCDF[b]	6	7.6	45
2,3,4,7,8-pentaCDF	44	50	130
Total pentaCDFs	130	150	490
1,2,3,7,8-pentaCDD	13	11	28
Total pentaCDDs	86	76	270
1,2,3,4,7,8-hexaCDF[c]	12	16	50
1,2,3,6,7,8-hexaCDF	3	5	10
1,2,3,7,8,9-hexaCDF	3	3	11
2,3,4,6,7,8-hexaCDF	16	18	63
Total hexaCDFs	70	88	280
1,2,3,4,7,8-hexaCDD	8	5	14
1,2,3,6,7,8-hexaCDD	26	18	71
1,2,3,7,8,9-hexaCDD	3	4	7
Total hexaCDDs	154	170	465
Total heptaCDFs	23	28	90
Total heptaCDDs	32	30	85
octaCDF	< 1	< 1	< 2
octaCDD	< 1	< 1	< 2

[a]   From: Rappe et al. (1987).

[b]   Not separated from 1,2,3,4,8-pentaCDF.

[c]   Not separated from 1,2,3,4,7,9-hexaCDF.

        Table 26. TCDD content of the livers of farm animals from Seveso

contaminated zones and surrounding areas (1976-1979)[a]

Animal	Number of samples	TCDD-containing samples	TCDD maximum level (ng/g)
Rabbits[b]	698	433	633
Poultry	83	35	24
Cattle	43	21	94
Horses	12	2	88
Pigs	13	0	–
Goats	25	17	1
Cats	1	0	–

[a] From: Pocchiari et al. (1983).
[b] Figures include rabbits kept in the special test plots on
     contaminated ground for experimental purposes.

   Table 27. CDD analyses of wildlife from Seveso contaminated zones and
surrounding areas (1976-1979)[a]

Animal	Tested organs and number of samples	Number of TCD-containing samples	Maximum level of TCDD (ng/g)
Rabbits	6 (liver)	4	13
Field mice	14 (whole body)	14	49
Rats	1 (pool-4 livers)		28
Earthworms	2 (pool)		12
Frogs	1 (liver)		0.2
Snakes	1 (liver)		3

[a]   From: Pocchiari et al. (1983).

Table 28. Analytical results for 2,3,7,8-tetraCDD residues[a]

Sections of 11 deer in study	No. of deer samples analyzed	No. of positive samples	Concentration range of TCDD detected (ng/kg)[b]	Limit of detection range (ng/kg)[b]
Muscle	11	3	12 – 27	0.5 – 5

| Adipose tissue | 10 | 8 | 3 - 12 | 1 - 3 |

Table 28. (cont'd) Analytical results for 2,3,7,8-tetraCDD residues[a]

Sections of 11 deer in study	No. of deer samples analyzed	No. of positive samples	Concentration range of TCDD detected (ng/kg)[b]	Limit of detection range (ng/kg)[b]
Liver	11	4	2 - 5	0.4 - 4
Bone marrow	5	0	ND	1 - 3

[a]   From: Harless et al. (1983).

[b]   Results corrected for efficiency of sample preparation.
       ND = not detected.

4.4.4.1 Adipose tissue

     Gross et al. (1984) reported a study in which 30 coded samples of
adipose tissue from Viet Nam veterans were analyzed for TCDD. The TCDD
levels found for two of the three heavily exposed men were 99 pg/g and
63 pg/g, which is higher than for the other Viet Nam veterans or for
the controls (all well below 15 pg/g). Only one single isomer of
tetraCDDs was found, and it was assumed that this was the
2,3,7,8-isomer. The data in this study has also been discussed by
Young et al. (1983). These authors concluded that the levels do not
correlate well with known exposure data or with health status.

     Rappe et al. (1984) reported the presence of 2,3,7,8-substituted
PCDDs and PCDFs in samples of human adipose tissue from Northern
Sweden. A series of reports presented during the period 1984-1986
confirms these observations and it has been clearly shown that there
is a background of 2,3,7,8-substituted PCDDs and PCDFs in the general
population in the industrialized part of the world. Most of these
reports are lacking data on how the sampled people were selected and
possible exposure to PCDDs and PCDFs. Consequently, these studies
might not be representative. A series of earlier studies failed to
detect these background levels due to insufficiently low detection
levels. The Swedish study included 31 people, of which 18 were exposed
to phenoxy esters and 13 were nonexposed. The group included 17 cancer
patients and 14 non-cancer patients. The different groups were matched
against each other. No difference in the levels, isomer patterns, or
ranges could be found between these subgroups (Nygren et al., 1986).

The mean values for these 31 people are given in Table 29.

Schecter et al. (1986a) reported the mean levels of PCDDs and PCDFs in 46 samples of adipose tissue collected in Canada and in 8 samples from the USA (see Table 29). The Canadian samples were taken from people who had died in 1976 from car accidents, drownings,

trauma, and suicide. The samples included all ages and both sexes and came from all over the country. The USA samples, (1983-1984), were taken from biopsies from New York State residents during the course of normal medical procedures, and also from autopsies. Table 29 also includes the PCDD and PCDF levels in adipose tissue samples from Viet Nam (Schecter et al., 1986b) and from cancer patients in Japan (Ono et al., 1986).

It is interesting to note the similarity between isomers present, levels of isomers, isomeric patterns, and congener profiles in samples collected from the general population in industrialized countries on three continents. The profile of the PCDD isomers shows increasing levels with an increasing number of chlorine atoms; the level of OCDD is 230-900 pg/g. On the other hand, the profile of PCDFs shows a maximum for 2,3,4,7,8-penta- or 1,2,3,6,7,8-hexaCDF. The difference in levels found between samples from South and North Viet Nam may be explained by spraying during the war in the 1960s and by the difference in industrial activities between the two parts of the country.

Four samples of adipose tissue taken from German workers exposed to TCDD in the early 1950s have also been analyzed (Rappe et al., 1987). In spite of the fact that these workers were exposed more than 30 years before collecting the samples, enhanced levels of 2,3,7,8-tetraCDD could be identified, but the levels of the other PCDDs and PCDFs seem to be in the normal range (Table 29, last column).

Patterson et al. (1986) studied the levels of 2,3,7,8-tetraCDD in the adipose tissue of 39 exposed people and 57 controls in Missouri, USA. The exposed group had subgroups of recreational, residential, and occupational exposure. All persons in both the exposed and control groups had detectable levels of 2,3,7,8-tetraCDD in their adipose tissue. Nineteen of the 39 exposed people had measurements higher than the highest level in the control group and six of the exposed people had levels greater than 100 ng/kg, which was five times higher than the highest control (Table 30).

Ryan et al. (1986) analyzed autopsy tissue samples that were

collected from three subjects who died in New York State, USA, from
natural causes. The tissue types were: fat (both abdominal and
subcutaneous), adrenal, bone marrow, liver, muscle, spleen, kidney,
and lungs. As far as could be ascertained, no subjects had known
abnormal exposure to PCDDs or PCDFs, yet these chemicals were found in
all tissues analyzed. The highest concentrations of all PCDDs and
PCDFs were found in adipose tissue. In individual tissues of the three
subjects, the levels of individual congeners detected were within a
narrow range, with much higher levels of the higher chlorinated PCDDs
and PCDFs (e.g. hepta- and octa-CDD). In adipose tissue, the level of
2,3,7,8-tetraCDD was 3.7-8.4 ng/kg and that of 2,3,4,7,8-pentaCDF

5.2-13 ng/kg, while octaCDD ranged between 430 and 700 ng/kg. No major
differences were seen between abdominal and subcutaneous fat samples
or between these two types and perirenal fat when the lower lipid
content of the latter was considered. Smaller concentrations of PCDDs
and PCDFs were measured (on a wet weight basis) in decreasing order:
adrenal, bone marrow, liver, muscle, spleen, kidney, and lungs.

     The high levels found in exposed Viet Nam veterans and German
workers indicate a very slow excretion rate or metabolism of TCDD in
humans. This indicates a dramatic difference between man and rodents;
in the latter the half-life of TCDD is reported to be in the range of
a few weeks.

4.4.4.2 Blood plasma

     Analysis of blood plasma has been used to evaluate occupational
exposure to PCDDs and PCDFs, which can occur during the production or
use of 2,4,6-tri-, 2,3,4,6-tetra-, and pentachlorophenol. Rappe et al.
(1983) investigated such exposure through the analysis of blood plasma
of exposed workers and unexposed controls. Good correlations were
found between the plasma levels and:

(a) the nature of exposure - dermal contact with liquids resulted in
     higher levels than inhalation of contaminated dust;

(b) the duration of exposure - higher levels for longer exposure
     times.

The isomers present in the formulations used could also be found in
the blood plasma.

     Kochman et al. (1986) studied a group of people exposed to PCBs
and PCDFs after a transformer fire in Rheims, France in 1985. Low
levels (1-25 pg/g) of 2,3,7,8-substituted penta-, hexa-, hepta-, and

octaCDFs were found in the blood plasma of these people, and there was a slight variation in the T-lymphocytes cells.

Kahn et al. (1986) measured the levels of PCDDs and PCDFs in the blood plasma from 10 heavily exposed Viet Nam veterans and their 17 controls. The levels of TCDD was much higher in the exposed men than in their controls.

Table 29. Levels of PCDDs and PCDFs in human adipose tissue (ng/kg wet weight)

Isomer	Canada[f] n=46[b]	Japan[f] n=13[c]	N Viet Nam[f][f] n=9[d]	Sweden n=31[a]	FRG n=8[b]	FRG n=15[d]	USA/NY[f] n=4[e]
2,3,7,8-tetraCDD	9 (12)	< 2	28 (12)	3	7.2	150	6.4 (25)
1,2,3,7,8-pentaCDD	15 (13)	< 2	15 (14)	10	11.1	19.2	10 (46)
1,2,3,6,7,8-hexaCDD	70 (12)	11 (6)	100 (15)	15	96	77	81 (46)
1,2,3,7,8,9-hexaCDD	12 (10)	NA	NA	4	NA	9.4	NA
1,2,3,4,6,7,8-heptaCDD	77 (12)	28 (6)	178 (15)	97	164	56	135 (46)
octaCDD	230 (12)	104 (8)	1256 (15)	414	707	267	830 (46)
2,3,7,8-tetraCDF	9 (13)	NA	NA	3.9	NA	0.9	NA
2,3,4,7,8-pentaCDF	25 (13)	13 (7)	21 (15)	54	14.3	44	15 (46)
1,2,3,4,7,8-hexaCDF	15 (11)	NA	NA	6	NA	10.0	NA
1,2,3,6,7,8-hexaCDF	14 (11)	13 (7)	58 (15)	5	31.3	6.7	16 (34)
2,3,4,6,7,8-hexaCDF	8 (3)	NA	NA	2	NA	3.8	NA
1,2,3,4,6,7,8-heptaCDF	(44)	NA	7 (3)	11	16.5	29 (15)	30 19.5

	octaCDF	4	NA	NA	NA	NA	NA	1

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n   = number of tissue samples.

NA = not analyzed.

[a]   Nygren et al. (1986).

[b]   Schecter et al. (1986a).

[c]   Ono et al. (1986).

[d]   Schecter et al. (1986b).

[e]   Refers to occupationally exposed workers. Rappe et al. (1987).

[f]   Mean values of positives. Number of positives within brackets.

Levels below the detection level (< 1.0 ng/kg) not included.

Table 30. Comparison of Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (ng/kg) in Adipose Tissue of Exposed and Control Groups[a]

		Exposed			
Variable	Controls	Total	Recreational	Residential	Occupational
Number of subjects	57	39	8	16	15
Arithmetic mean	7.4	79.7	90.8	21.1	136.2
Median	6.4	17.0	23.5	14.5	24.7
Range	1.4–20.2	2.8–750	5.0–577	2.8–59.1	3.5–750
Geometric mean	6.4	21.8	24.8	15.3	29.8
Mean age, years (SD)	52.6 (15.7)	44.3 (13.7)	42.1 (14.7)	39.7 (14.9)	50.3 (9.8)
% Men	35.1	61.5	37.5	43.8	93.3

[a]   From: Patterson et al. (1986).

5.   ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Air

     Owing to sampling and analytical problems, very few data are
available on the levels of 2,3,7,8-TCDD and other PCDDs and PCDFs in
normal urban air.

     Rappe & Kjeller (1987) reported the levels of PCDDs and PCDFs in
total air samples collected in Hamburg, FRG, and samples of air
particulates collected in Sweden (Table 31). Sample 1 was collected on
the outskirts of Hamburg (representing an urban area), sample 2 in a
traffic tunnel, sample 3 downwind from a MSW incinerator, and sample
4 in the vicinity of a dumpsite and metal refinery. PCDDs and PCDFs
were found in all samples. Lower levels of PCDDs and PCDFs were found
in air particulates than in total air samples (Table 31). Sample 5 was
taken when "clean air" was blowing into a street in Gothenburg,
Sweden, and sample 6 was taken in the same street during an inversion
situation. Samples 7 and 8 were taken at a rural research station
outside Gothenburg when the air was blowing from the sea (sample 7) or
from Gothenburg (sample 8). The isomeric pattern found in these
samples were very similar (Rappe & Kjeller, 1987).

     Airborne dust was monitored in 1977 in the Seveso area to
evaluate the possibility that 2,3,7,8-tetraCDD-contaminated particles
might have drifted outside the contaminated areas. A high-volume
sampling technique was used. When pooled particulate samples were
analyzed, levels of 0.17-0.50 pg TCDD/m3 were reported (Wipf et al.,
1982).

     The atmospheric concentrations of 2,3,7,8-tetraCDD near two
hazardous waste sites have been monitored. In one study, US EPA (1982)
failed to detect (detection limit: 1-20 pg/m3) any 2,3,7,8-TCDD in the
atmosphere at the Love Canal (New York, USA) area. In another study of
a waste disposal site (near Jacksonville, Arkansas, USA), Thibodeaux
(1983) reported an average concentration of 1100 pg of 2,3,7,8-TCDD/g
in two air particulate samples collected near the disposal site.

     Rappe et al. (1985c) analyzed indoor air samples for PCDFs
resulting from fires and explosions in PCB-filled electrical
equipment, and in an industrial situation (locomotive shop) (Table
32).

O'Keefe et al. (1985) analyzed air samples collected in an office building in Binghamton, New York, USA, after a transformer accident in the basement in February 1981. The samples were collected after a primary clean-up and the values are given in Table 33.

Table 31. Levels of PCDDs and PCDFs in samples of total air and air particulates[a]

Total air samples	Air particulates							
	1 pg/m^3	2 pg/m^3	3 pg/m^3	4 pg/m^3	5 fg/m^3	6 fg/m^3	7 fg/m^3	8 fg/m^3
2,3,7,8-tetraCDF[b]	0.04	0.72	0.38	0.18	30	240	5	62
Total tetraCDFs	0.36	6.2	4.9	3.3	320	2000	54	490
2,3,7,8-tetraCDD	0.02	0.06	0.02	0.08	3	9	< 1	5
Total tetraCDDs	0.10	0.22	0.21	1.5	150	350	9	130
1,2,3,7,8-pentaCDF[c]	0.04	0.36	0.42	1.0	39	190	7	58
2,3,4,7,8-pentaCDF	0.04	Int[e]	0.43	1.2	51	240	6	69
Total pentaCDFs	0.51	4.1	5.0	10	470	2500	85	610
1,2,3,7,8-pentaCDD	< 0.02	0.28	0.22	0.6	17	66	5	35
Total pentaCDDs	0.07	1.3	2.4	5.0	200	840	31	280
1,2,3,4,7,8-hexaCDF[d]	0.03	0.13	0.27	1.1	23	100	8	38
1,2,3,6,7,8-hexaCDF	0.03	0.15	0.24	1.4	20	78	8	33
1,2,3,7,8,9-hexaCDF	< 0.01	< 0.05	< 0.02	0.33	4	17	3	14
2,3,4,6,7,8-hexaCDF	< 0.01	< 0.05	0.12	0.80	10	84	7	32
Total hexaCDFs	0.18	1.1	2.2	9.5	180	800	70	310
1,2,3,4,7,8-hexaCDD	< 0.08	< 0.17	0.19	1.0	3	19	< 1	7
1,2,3,6,7,8-hexaCDD	0.23	0.66	0.71	2.2	11	46	4	14
1,2,3,7,8,9-hexaCDD	< 0.08	< 0.17	0.36	5.2	6	92	5	32
Total hexaCDDs	0.74	2.7	5.3	24.	100	520	32	190
Total heptaCDFs	0.10	1.2	2.0	5.	200	1100	120	500
Total heptaCDDs	0.60	3.4	5.3	15.	380	2900	140	1000

OctaCDF	< 0.11	< 1.0	0.78	7.0	150	480	100	440
OctaCDD	0.37	6.4	7.4	40.0	290	1900	64	540

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a From: Rappe & Kjeller (1987).
b Not separated from 2,3,4,8-tetraCDF.
c Not separated from 1,2,3,4,8-pentaCDF.
d Not separated from 1,2,3,4,7,9-hexaCDF.
e Int = Interferences.

5.2 Water and Leachate

Shadoff et al. (1977) failed to identify 2,3,7,8-tetraCDD in water from areas in the USA where 2,4,5-T herbicides had been used.

In the 2,4,5-T plant in Jacksonville, Arkansas, USA, Thibodeaux (1983) could not detect any 2,3,7,8-tetraCDD in the creek water (no limit of detection levels given).

Since August 1976, a number of tests have been periodically conducted in Seveso on streams running through the affected area, as far south as the River Lambro, with consistently negative results. During the same period sediment samples were taken from Torrents, Certesa, and Seveso. Positive results of the order of 1 pg/g were obtained within the first few kilometers downstream from their confluence, but further downstream results were negative. The intensive rainfalls after the accident caused the Seveso to repeatedly overflow its embankments at the point of entry into Milan, thus depositing silt on adjacent areas. Tests conducted to determine TCDD in these silts yielded negative findings for the first four floods while the fifth flood yielded positive findings (pg/g). Since August 1976, the monthly determinations conducted on pipeline and ground waters have consistently yielded negative results, even when the analytical detection threshold was as low as 1 pg/litre (parts per quadrillion) (Pocchiari, 1983).

During 1983 and 1984, the Dow Chemical Company conducted a study to determine the 2,3,7,8-tetraCDD contamination at its plant in Midland, Michigan, USA. It was estimated that 0.6 g of 2,3,7,8-tetraCDD was being emitted per year in 2.5×10^7 m^3 of wastewater effluent (Lamparski et al., 1986).

The Ontario Ministry of Environment has included PCDDs and PCDFs in its Drinking Water Surveillance Program for the St Clair/Detroit

River area (Ontario, 1986). No 2,3,7,8-tetraCDD has been found in any sample of raw or treated water. Unspecified congeners of PCDDs and PCDFs have been found mainly in raw water, octaCDD being the most frequent congener found. The highest value reported was 1.1 pg octaCDD/litre raw water in Amherstburg. The octaCDD level in the treated water was below the detection level of 0.01 pg/litre.

Götz (1986) reported on levels of PCDDs and PCDFs in the oily leachate from a sanitary landfill in Georgswerder, Hamburg, FRG (table 34).

5.3 Soil and Sediment

The levels of 2,3,7,8-tetraCDD in point source after improper disposal of industrial waste are discussed in section 3.4.2.

Table 32. Analyses of PCDFs in air samples (pg/m3)[a]

Sample	total tetra- CDF	2,3,7,8- tetraCDF	penta- CDF	hexa- CDF	hepta- CDF	octa- CDF
Surahammar (during cleaning)	< 20	< 2	< 10	< 10	< 10	< 10
Surahammar (after cleaning)	< 10	< 2.5	< 10	< 10	< 10	< 10
Railway locomotive (during cleaning operations)	500	50	50	30	20	20

[a]   From: Rappe et al. (1985c).

Table 33. Concentrations of PCDFs in air samples collected on various floors of a Binghamton, New York (USA) office after primary cleanup

	Analytical results (pg/m^3)			
Floor/sample type[a]	2,3,7,8- tetraCDF	Total tetraCDFs	Penta- CDFs	Hexa- CDFs

3	16	151	43	
5	11	126	30	2.0
5 (NE)	20	195	60	8.7
7	11	121	36	
9 volatiles	14	140	42	
9 particulates	1.8	4.8	4.7	
9 (SE) volatiles	13	146	31	3.7
9 (SE) particulates	0.8	3.9	3.2	
11	23	76	16	
11 (SE + NW)	16	133	19	
14	11	92	21	
14 (NE)	14	185	13	
16	16	118	21	
17 volatiles	12	79	24	
17 particulates	0.8	3.9	ND[b]	
17 volatiles	9	59	6.6	
17 particulates	0.9	ND[b]	2.9	

Table 33. cont'd

[a]   Abbreviations in parentheses designate sampling location on the floor,
      e.g., SE = south-east corner. Unless otherwise specified samples were
      collected in the north-west corner and analyzed as combined particulates
      and volatiles.

[b]   ND = not detected.

Table 34. Levels (ng/g) of the 2,3,7,8-substituted PCDDs and PCDFs in
          leachate from a sanitary landfill[a]

Isomer	Concentration	Isomer	Concentration
2,3,7,8-tetraCDD	60	2,3,7,8-tetraCDF	9
1,2,3,7,8-pentaCDD	28	1,2,3,7,8-pentaCDF[b]	322
1,2,3,4,7,8-hexaCDD	476	2,3,4,7,8-pentaCDF	261
1,2,3,6,7,8-hexaCDD	1440	1,2,3,4,7,8-hexaCDF	748
1,2,3,7,8,9-hexaCDD	310	1,2,3,6,7,8-hexaCDF	336
		1,2,3,7,8,9-hexaCDF	558
		2,3,4,6,7,8-hexaCDF	114

a   From: Götz (1986).

b   Overlapping isomer: 1,2,3,4,8-pentaCDF.

Analytical results of the 1976-1977 survey of Zones B and R in Seveso were discussed by Pocchiari (1983). TCDD levels in Zones B and R were, in general, considerably lower than those in Zone A. In fact, most TCDD levels were lower than 50 $\mu g/m^2$ in Zone B and 5 $\mu g/m^2$ in Zone R. In 1980, a large part of Zone R was remonitored to evaluate the persistence of TCDD in the soil. This zone had been ploughed and worked since 1978. A comparison, as well as a statistical evaluation, of the relevant data indicated a significant decrease (40%) in the geometric mean level of TCDD in the soil of Zone R.

In 1980 and 1981, soil samples from ten sites in Zone R and five sites outside Zone R were analyzed using a high resolution GS-MS system to establish whether other isomers of 2,3,7,8-tetraCDD were also present. A significant percentage decrease in tetraCDDs could be accounted for by two isomers (1,3,6,8-tetraCDD and 1,3,7,9-tetraCDD) present in the majority of the samples tested. These two isomers were not related to the chemical accident at the factory.

Table 35. PCDD contamination in soil from Zone R in Seveso (1981) (values in ng/kg)[a]

Sample	2,3,7,8[b] tetraCDD	TCDD[c]	Penta- CDDs	Hexa- CDDs	Hepta- CDDs	Octa- CDD	Total PCDDs
S1	0.8	0.3	0.4	6.0	1.4	1.7	10.6
S2	3.4	1.0	0.7	9.5	2.1	2.2	18.9
S3	4.0	1.9	1.2	8.2	8.6	27.0	50.9
S4	2.3	0.8	0.6	10.2	2.1	2.0	18.0
S5	< 0.1	< 0.3	0.5	9.5	1.9	1.4	13.7
S6	6.3	1.5	1.1	10.4	2.6	1.3	24.8
S7	1.7	1.0	0.8	12.4	1.9	1.8	19.6
S8	2.2	0.4	0.8	8.8	1.8	0.8	14.8
S9	1.0	2.8	2.3	21.2	9.6	13.5	50.4

a   From: Wipf & Schmid (1983).

b   Probably related to the accident.

c   Total levels of isomers other than 2,3,7,8-tetraCDD, probably not related to accident.

Wipf & Schmid (1983) reported the presence of PCDDs other than 2,3,7,8-tetraCDD in the soil from Zone R (Table 35). They suggest that a municipal incinerator and the burning of wood shavings treated with chlorinated phenols could be the source of the other PCDDs.

Nestrick et al. (1986) reported the levels of 2,3,7,8-tetraCDD in soil samples collected from industrialized areas of US cities. They observed a widespread occurrence of 2,3,7,8-tetraCDD in urban soils, with levels of 1-10 ng/kg, and suggested that local combustion sources, including MSW and industrial incinerators, were the probable origin.

McLaughlin & Pearson (1984) measured soil concentrations of PCDDs and PCDFs in the vicinity of a municipal refuse incinerator in Ontario, Canada. Urban and rural control locations were also sampled. All soil samples (14) had detectable quantities of at least one of the five PCDD congener classes (tetraCDD to octaCCD) tested for, whereas eight samples contained detectable levels of one or more of the five PCDF congener groups (tetraCDF to octaCDF). The levels ranged from non-detectable (0.003 - 0.008 ng/g) to 3.5 ng/g (octaCDD); only one site had a measurable quantity (0.007 ng/g) of tetraCDD in the soil. The most abundant PCDD or PCDF congener was octaCDD, which had similar levels whether samples were taken close to or remote from the incinerator. Similarly, no concentration gradients, relative to distance from the incinerator, were apparent for any of the other PCDDs or PCDFs.

Soil samples near a chemical waste incinerator in Scotland have also been analyzed for PCDDs and PCDFs, together with samples from control locations (Edulgee et al., 1986). Detectable levels of all PCDFs or PCDFs that were examined were found in each of the soil samples (13). Levels found ranged from 1.2 ng/kg (2,3,7,8-tetraCDD) to 1900 ng/kg (total hexaCDF). No consistent pattern was observed to differentiate levels found in control samples from levels in soil near the incinerator.

These studies from widely separate areas of the world support the suggestion that diffuse combustion sources are the major source of PCDDs and PCDFs in the soil.

Rappe & Kjeller (1987) analyzed soil samples from various parts of Europe (Table 36). They represent rural areas (samples 1, 2, and 3) as well as more industrialized areas (samples 4 and 5). PCDDs and PCDFs could be identified in all samples. The 2,3,7,8-tetraCDD concentration was below the detection level in the soil samples from

the non-industrialized areas. Trapped sediments from the archipelago of Stockholm, Sweden, were also analyzed. The samples were collected in the inner (sample 6), middle (sample 7), and outer archipelago (sample 8). Levels decreased with increasing distance from the city of Stockholm. The isomeric patterns for tetra- and pentaCDF isomers are very similar to those found for samples of total air and air particulates (section 6.1). A sediment sample from the mouth of River Viskan, Sweden, was also analyzed (sample 9). A slight difference in congener profile was found between this sample and the sediments from the archipelago of Stockholm.

Czuczwa & Hites (1985) found PCDDs and PCDFs in sediment samples from several locations in Saginaw River and Bay, and southern Lake Huron, levels ranging from 100 ng/g in urban areas to 100 ng/kg at remote sites. Although no isomers were identified, the analytical profiles in the sediments followed closely those found in combustion samples, suggesting that combustion is the major source of PCDDs and PCDFs found in the sediments. Analyses of sediment cores showed a dramatic increase in the PCDD and PCDF concentrations at a depth corresponding to approximately the year 1940, and levels remained high up to the present. There is no good correlation between the trend in these levels and the trend for coal burning in the United States. However, the levels in the sediments correlate with the production and use of chlorinated aromatic compounds within this area of the Great Lakes.

5.4 Food

5.4.1 Meat and bovine milk

The levels of PCDDs and PCDFs in fish and other seafood are discussed in section 4.4.2.

Table 36. Levels (pg/g) of PCDDs and PCDFs in samples of sediments and soil[a]

samples				Soil Sediments			
			1	2	3	4	
5	6	7	8	9			
2,3,7,8-tetraCDF[b]			2.9	1.6	1.1	34	
38	30	17	14	1.6			
Total tetraCDFs			9.3	7.7	11	320	

	370	290	150	120	24				
2,3,7,8-tetraCDD	< 2.0	< 2.1	< 0.2	2.4	0.8	2.4	2.0	< 2.0	0.2
Total tetraCDDs	–	–	3.2	55.5	11.2	69	21	23	6.4
1,2,3,7,8-pentaCDF[c]	2.5	1.6	0.5	17	31	16	8.6	8.5	1.3
2,3,4,7,8-pentaCDF	0.8	1.0	0.6	23	65	20	14	16	1.7
Total pentaCDFs	14	13	6.7	200	450	260	140	130	30
1,2,3,7,8-pentaCDD	< 2.0	< 2.0	< 0.1	18	34	7.6	5.2	5.5	0.9
Total pentaCDDs	–	–	4.6	220	270	230	99	86	13
1,2,3,4,7,8-hexaCDF[d]	3.8	2.2	0.9	30	45	16	10	8.8	1.9
1,2,3,6,7,8-hexaCDF	1.8	1.5	0.4	11	25	12	7.1	5.9	1.2
1,2,3,7,8,9-hexaCDF	1.0	0.9	4.3	110	1100	5	< 1	< 1	2.0
2,3,4,6,7,8-hexaCDF	1.9	1.0	0.7	26	57	16	31	23	1.6
Total hexaCDFs	16	12	11	270	1900	250	220	92	44
1,2,3,4,7,8-hexaCDD	< 2	< 2	< 0.1	13	28	1.6	0.8	1.0	1.6
1,2,3,6,7,8-hexaCDD	< 2	< 2	< 0.1	19	64	48	2.0	2.0	10
1,2,3,7,8,9-hexaCDD	< 2	< 2	< 0.1	6.2	19	2.5	0.9	1.0	4.3
Total hexaCDDs	–	–	4.7	200	330	49	16	19	64
Total heptaCDFs	22	14	18	260	4500	1300	1500	190	300
Total heptaCDDs	< 10	< 10	17	370	1600	5700	1200	880	190
OctaCDF	–	–	5.7	68	71	39	< 20	< 20	330
OctaCDD	–	–	14	140	180	3100	510	260	900

a   From: Rappe & Kjeller (1987).

b   Not separated from 2,3,4,8-tetraCDF, except in the case of sample 9.

c   Not separated from 1,2,3,4,8-pentaCDF.

d   Not separated from 1,2,3,4,7,9-hexaCDF.

    The US Environmental Protection Agency (US EPA) initiated a
2,3,7,8-tetraCDD monitoring programme of beef fat samples taken from
cattle that had grazed on rangelands known to have been treated with
2,4,5-T. The analytical collaborators in this programme were Dow
Chemical Co. (USA), Wright State University, and Harvard University.
All the laboratories used mass spectroscopic techniques for
quantification. Two different extraction techniques were used. All
three laboratories analyzed control samples taken from cattle that had
grazed on non-treated areas. Some of these control samples were spiked
with known amounts of TCDD. All the controls were prepared by the US
EPA (Firestone, 1978). Good agreement was found between the amounts of
TCDD spiked into the control beef fat samples and the reported levels
found, even down to TCDD levels of 10 ng/kg. The average reported TCDD
level was 10 ng/kg; the amount actually added by the EPA was 9 ng/kg.
Of a total of 34 analyses of controls to which no TCDD was added, in
only one case was there a false positive report of TCDD (O'Keefe et
al., 1977). Of 52 samples of beef fat from 2,4,5-T-treated rangeland,
19 (37%) were reported by one or more laboratories to have TCDD. The
average range of levels reported was 5-66 ng/kg, and the overall
average was 7 ng/kg. If one considers only the 40 beef fat samples
from areas receiving at least 1.1 kg of 2,4,5-T/ha, all 19 positive
samples (48%) belong to this group, and the average reported TCDD
level would be 9 ng/kg. The results indicated a consistent trend,
relating the average reported TCDD level in beef fat to the intensity
of the 2,4,5-T application to rangeland (O'Keffe et al., 1978;
McKinney, 1978).

    None of the three collaborating laboratories used the most
selective and sensitive analytical method now known (capillary glass
column gas chromatography - high resolution mass fragmentography).

    Kocher et al. (1978) also analyzed specimens of fat taken from
steers that had grazed on rangeland previously treated with 2,4,5-T
herbicides. The limit of detection of TCDD (2.5 times peak to peak
noise) was found to be in the 30-60 pg range (3-6 pg/g in beef fat
using 10 gram samples). None of the sixteen samples analyzed in two of
three studies revealed TCDD. In the third study, the animals were

confined to a fenced pasture sprayed in its entirety with 2,4,5-T
herbicides. The samples from three of the seven animals gave a
positive response at the extremely low level of 3 to 4 ng TCDD/kg,
which is at the detection limit; the highest reported value (without
interfering components) was 13 pg/g. The level of 2,3,7,8-tetraCDD in
the 2,4,5-T used was, however, unknown. Beck et al. (1986) analyzed
seven randomly collected samples of cow's milk from different areas of
the Federal Republic of Germany and one sample from the German
Democratic Republic. The cow's milk was taken from road transport
tankers. In all samples, 2,3,7,8-substituted PCDDs and PCDFs were
found at low levels of pg/g on a fat weight basis. Detection limits

were in the range 0.1-0.3 ng/kg. The levels of PCDDs and PCDFs found
in the milk samples were lower than those measured by Rappe et al.
(1987b) in cow's milk from Switzerland (Table 38). In addition, there
was no evidence of high levels of the higher chlorinated congeners
(e.g. hepta and octa-) (see Table 37). Levels were much lower than
those in human milk samples (see section 5.4.2).

     Rappe et al. (1987b) analyzed PCDDs and PCDFs in six samples of
bovine milk from various locations in Switzerland. In all samples,
2,3,7,8-substituted PCDDs and PCDFs were found at levels of pg/kg in
whole milk (Table 38). However, the levels were lower in commercial
milk samples than in samples collected directly from cows grazing in
the vicinity of incinerators.

     When Ryan et al. (1985) analyzed PCDDs and PCDFs in chicken and
pork samples in Canada, the incidence of positives for hexa-, hepta-,
and octaCDD in selected samples of chicken fat was 50, 62, and 46%,
with averages of 27, 52, and 90 ng/kg, respectively. Similar levels of
hexa- and heptaCDFs were also found in some of these samples, but
tetra- and pentaCDDs and tetra-, penta-, and octaCDFs were not
detected. A comparison between the tissue analyses and those of the
wood (treated with pentachlorophenol) used to house the animals showed
a marked similarity (see Table 38), indicating that pentachlorophenol
was the probable source of contamination of the food samples.

     Firestone et al. (1986) reported the analyses of various food
items collected in the period beginning in 1979. Low levels (< 300
ng/kg) of 1,2,3,4,6,7,8- and 1,2,3,4,6,7,9-heptaCDD were found in some
samples of chicken, bacon, pork chops, and beef liver. HexaCDD was not
found in any of the foods. Several beef livers had high levels of OCDD
residues, the highest reported value being 3830 ng/kg. No PCDDs (at a
detection limit of 10-40 ng/kg) were found in ground beef.

5.4.2 Human milk

Rappe et al. (1984) reported low levels of 2,3,7,8-substituted PCDDs and PCDFs in five samples of human milk from the Federal Republic of Germany (FRG) (Table 39).

Table 39 also indicates the results of analyses of four samples of human milk from the Umea region of northern Sweden (Rappe, 1985), 92 samples from Rheinland-Westfalia in the FRG (Furst et al., 1987), 30 other samples from the FRG (Beck et al., 1987), and five samples from the Netherlands or Yugoslavia (Rappe et al., 1987).

Van der Berg et al. (1986) also reported the levels of PCDDs and PCDFs in human milk samples from the Netherlands, but these levels were reported on milk basis, not on fat basis, and are therefore not included in Table 39.

A comparison between the isomers, levels, isomeric pattern, and congener profiles found in human milk (Table 39) and in adipose tissue (Tables 29 and 30) shows a remarkable degree of similarity.

5.4.3 Rice

Rice from fields in Arkansas, Louisiana, and Texas, USA, treated at a maximum rate to give 2.52 kg 2,4,5-T/ha, were analyzed for possible 2,3,7,8-tetraCDD residues. A specification of 1 µg 2,3,7,8-tetraCDD/g 2,4,5-T was given in the report, but no analytical data were given for the herbicide. No 2,3,7,8-TCDD was detected in the rice (detection limit = 2-7 µg/kg) and no 2,3,7,8-TCDD residues (detection limit = 2-10 µg/kg) were found in 30 samples of rice purchased in retail stores throughout the USA (Jensen et al., 1983).

5.5 Yusho and Yu-cheng Episodes

In 1968, more than 1500 people in south-west Japan were intoxicated through consuming commercial rice oil accidentally contaminated by PCBs, PCDFs, and polychlorinated quarterphenyls (Masuda & Yoshimura, 1982; Masuda et al., 1985). In 1979, a similar episode occurred in central Taiwan, the number of persons involved here approaching 2000 (Chen et al., 1980; Chen et al., 1985). In the past, both accidents were referred to as Yusho episodes, but now the Taiwan episode has been renamed Yu-cheng.

The Japanese rice oil contained more than 40 PCDF isomers (tri- to hexaCDFs) (Buser et al., 1978d), whereas the number of isomers in the Taiwanese oil seems to have been less (Chen & Hites, 1983). The toxic 2,3,7,8-substituted PCDFs were middle or minor constituents,

about 10-15% of the total amount of PCDFs (Buser et al., 1978d; Masuda et al., 1985; Chen & Hites, 1983). The mean total consumption of PCDFs of the Yusho and Yu-cheng patients has been estimated to be 3.3-3.8 mg/person (Hayabuchi et al., 1979; Chen et al., 1985), or a daily intake of total PCDFs of 0.9 µg/kg body weight (Hayabuchi et al., 1979). The average intake of 2,3,7,8-substituted PCDFs was 90-135 ng/kg body weight per day. The smallest amount of total PCDFs causing chloracne has been estimated to be 0.16 µg/kg body weight per day (Hayabuchi et al., 1979) or 20-30 ng/kg per day of the 2,3,7,8-substituted congeners.

Analysis of liver samples taken from the Yusho patients about 18 months after the exposure showed a dramatic decrease in the number of PCDF isomers. Apparently most of the PCDF isomers were metabolized or excreted during the period between exposure and sampling (Rappe et al., 1979). A comparison between the PCDF isomers found in the Yusho oil and the liver samples revealed an interesting relationship. Most of the isomers retained had lateral positions (2, 3, 7, and 8) substituted with chlorine; these isomers have the highest toxicity (Rappe et al., 1979).

Table 37. PCDD and PCDF levels in samples of cow's milk (ng/kg on a fat weight basis)[a]

SAMPLE

	1	2	3	4	5	6	7	8
2,3,7,8-tetraCDF	< 0.1	0.29	0.28	1.1	1.0	1.4	1.4	0.27
2,3,7,8-tetraCDD	0.33	< 0.2	< 0.2	< 0.2	ND	< 0.2	ND	ND
1,2,3,7,8-pentaCDF	ND	0.4	ND	0.26	0.24	ND	0.39	< 0.2
2,3,4,7,8-pentaCDF	1.3	0.91	1.1	1.6	1.5	1.3	2.9	0.8
1,2,3,7,8-pentaCDD	1.0	0.72	ND	0.81	0.6	0.78	1.2	< 0.5
1,2,3,4,7,8-hexaCDF	0.93	0.67	0.70	0.85	< 0.3	0.84	1.9	0.57
1,2,3,6,7,8-hexaCDF	0.73	0.58	0.57	0.85				

				< 0.3	0.73	2.1	0.41
2,3,4,6,7,8-hexaCDF	0.65	0.48	0.53	0.68	ND	0.64	1.8	0.37
1,2,3,4,7,8-hexaCDD	0.33	0.34	< 0.3	< 0.3	< 0.3	0.36	0.33	< 0.3
1,2,3,6,7,8-hexaCDD	1.3	1.2	1.0	0.82	0.32	1.7	1.9	0.80
1,2,3,7,8,9-hexaCDD	< 0.3	0.34	0.36	0.39	ND	0.55	0.48	< 0.3
1,2,3,4,6,7,8-heptaCDF[b]	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
1,2,3,4,6,7,8-heptaCDD[b]	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
octaCDD[b]	<10	<10	<10	<10	<10	<10	<10	<10
octaCDF[b]	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1

[a]   From: Beck et al. (1987).

[b]   Not significantly higher than blanks.

ND   = not detectable.

Table 38. PCDF and PCDD content of bovine milk from Switzerland. Results in ng/kg (ppt), whole milk basis[a]

Compound	Commercial	Incinerators					
		1	2	3	4	5	6
2,3,7,8-tetraCDF		< 0.028	< 0.035	< 0.021	< 0.022	< 0.032	< 0.028
1,2,3,7,8-pentaCDF		< 0.020	< 0.022	< 0.021	< 0.020	< 0.036	< 0.032
2,3,4,7,8-pentaCDF		0.084	0.066	0.069	0.43	0.22	0.23
1,2,3,4,7,8-hexaCDF		< 0.020	< 0.026	< 0.017	0.13	0.06	0.084
1,2,3,6,7,8-hexaCDF		0.028	< 0.018	< 0.021	0.19	0.095	0.059
2,3,4,6,7,8-hexaCDF		< 0.020	< 0.018	ND	0.28	0.12	0.049 (< 0.02)
1,2,3,4,6,7,8-heptaCDF		< 0.12		ND			

```
ND                         0.49          0.28          < 0.18
                                                        (< 0.13)        (< 0.08)
                    octaCDF         < 0.20            ND
ND                     ND             ND          < 0.52
                                                        (< 0.13)        (< 0.09)
(< 0.16)        (< 0.21)

          2,3,7,8-tetraCDD          ND                ND
ND                     0.049        0.038          0.021
                                    (< 0.012)      (< 0.013)       (< 0.013)
        1,2,3,7,8-pentaCDD          ND                ND
ND                     0.25       < 0.086           ND
                                    (< 0.04)        (< 0.08)
(< 0.06)                                            (< 0.1)
      1,2,3,4,7,8-hexaCDD       < 0.068             ND
ND                     0.23         0.14         < 0.14
                                                        (< 0.1)         (< 0.06)
      1,2,3,6,7,8-hexaCDD       < 0.068             ND
ND                     0.29         0.16         < 0.21
                                                        (< 0.1)         (< 0.06)
      1,2,3,7,8,9-hexaCDD       < 0.068             ND
ND                     0.17       < 0.080        < 0.11
                                                        (< 0.1)         (< 0.06)
    1,2,3,4,6,7,8-heptaCDD      < 0.064           < 0.066          <
0.064                  0.26       < 0.095          0.42
                    octaCDD       < 0.16           < 0.26          <
0.12                   0.28       < 0.16           0.59
```

```
    a    From: Rappe et al. (1987b).
   ND    = not detectable.
```

Table 39. Levels of PCDDs and PCDFs found in human milk (ng/kg of fat weight).

			Sweden n=4[a]	FRG n=5[b]	FRG n=92[c]	FRG n=30[d]	Netherlands n=3[e]	Yugoslavia n=2[e]
2,3,7,8-tetraCDD			0.6	1.9	< 5	3.4	9.7	< 1.0
1,2,3,7,8-pentaCDD			6.5	12.9	10.7	15	44	5.5

1,2,3,4,7,8-hexaCDD	2.5	4.6	8.1	12	25	3.5
1,2,3,6,7,8-hexaCDD	19	17.3	32.7	59	251	15
1,2,3,7,8,9-hexaCDD	6.3	1.6	6.4	11	23	ND
1,2,3,4,6,7,8-heptaCDD	59.5	72.8	49.9	61	130	106
octaCDD	302	434	181	530	744	106
2,3,7,8-tetraCDF	4.2	5.4	2.6	2.5	2.8	< 1.0
1,2,3,7,8-pentaCDF	< 1	< 1	1.8	< 1	ND	ND
2,3,4,7,8-pentaCDF	21.3	36.4	22.9	20	79	25
1,2,3,4,7,8-hexaCDF	4.7	11.4	8.2	8.5	8.9	3.7
1,2,3,6,7,8-hexaCDF	3.4	10.2	6.6	7.8	10.3	3.6
2,3,4,6,7,8-hexaCDF	1.4	4.3	3.3	3.0	6.4	1.3
1,2,3,4,6,7,8-heptaCDF	7.4	9.2	6.4	8.5	39	ND
octaCDF	3.2	2.4	22.8	< 3	ND	ND

ND   = not detected; NA = not analyzed; n = number of samples.

[a]   Rappe (1985).

[b]   Rappe et al. (1984).

[c]   Furst et al. (1987).

[d]   Beck et al. (1987).

[e]   Rappe et al. (1987) (pooled samples)

Kunita et al. (1984) studied the blood levels of PCDFs in people involved in the Japanese and Taiwanese episodes. They used a non-isomer-specific analytical method, and reported higher blood levels in persons with severe dermal symptoms than in persons with light symptoms. The levels 2 years after exposure were lower than 0.5 year after exposure; for the six persons studied the levels of total

PCDFs decreased on average by 57% ± 12% (Kunita et al., 1984).

The rate of excretion of these toxic PCDF isomers is very low. Rappe & Nygren (1984) could detect 2,3,4,7,8-pentaCDF in blood plasma from Yusho patients when the samples were collected 11 years after exposure. However, higher levels were found in blood from Yu-cheng patients one year after exposure; these analyses also showed a 15-20% reduction in levels in one year (Rappe, 1984).

6.   KINETICS AND METABOLISM OF 2,3,7,8-TETRACHLORO-DIBENZO-P-DIOXIN (TCDD) AND OTHER PCDDs

6.1 Uptake, Distribution, and Excretion

Most of the available toxicokinetic data arise from studies of gastrointestinal exposure and oral or intraperitoneal administration. There has been one dermal study on rats, but no studies on exposure via the respiratory tract. Data on the gastrointestinal absorption, distribution, and elimination of TCDD in various species are summarized in Tables 40 and 41. Principle organ depots in all species studied are the liver and adipose tissue. In addition skin and muscle have been found to be organ depots in monkeys, and skin in guinea-pigs. In all species studied clearance of TCDD from the body follows apparent first-order kinetics.

6.1.1 Studies on rats

Male Sprague Dawley rats were dosed by gavage with 14C TCDD in acetone: corn oil (1:9), at a concentration of 50 µg/kg body weight (Piper et al., 1973). Rats lost weight and their physical condition was generally poor, although no deaths occurred. Approximately 30% of the administered dose of radioactivity was eliminated in the faeces during the first 48 h, this being most probably unabsorbed TCDD. The total faecal TCDD content was equivalent to 53.2% of the dose over a 21-day period. During this time 13.2% was eliminated in the urine and 3.2% in the air. From these results the half-time for the clearance of TCDD was calculated as 17.4 (± 5.6) days. In another study, groups of two to three rats were killed at different time intervals after administration of the same dose of ^{14}C-TCDD. The liver contained 3.2, 4.5, and 1.3% of the administered dose per gram of tissue 3, 7, and 21 days, respectively, after dosing. The concentrations in adipose tissue at the same time intervals were 2.6, 3.2, and 0.4% of the dose per gram of tissue. Other tissues showed lower concentrations.

In a study by Allen et al. (1975), male Sprague Dawley rats were given ^{14}C-TCDD in a single dose of 50 µg/kg body weight by stomach

tube. Groups of five rats were sacrificed 1, 3, 5, 7, 14, and 21 days
after dosing. The dose given resulted in marked liver hypertrophy,
thymic regression, weight loss, and death in 50% of the animals within
25 days. Twenty-five percent of the dose was eliminated within the
first 3 days through the faeces. During the next 18 days, 1 to 2% of
the dose was found in the faeces daily. The total amount in the faeces
during the 21 days following administration was about 52%.

Table 40. Gastrointestinal absorption of TCDD

Species/Strain	Vehicle Dose (µg/kg body weight)	% Absorption (mean ± SD)	Reference
Rats			
Sprague Dawley	acetone:corn oil (1:9) 50[a]	70	Piper et al. (1973)
Sprague Dawley	corn oil 50[a]	> 75	Allen et al. (1975)
Sprague Dawley	diet 42 days	7 or 20; for 50-60	Fries & Marrow (1975)
Sprague Dawley	acetone:corn oil (1:24) 1[a]	84±11	Rose et al. (1976)
Sprague Dawley	acetone:corn oil (1:24) 0.1 or 1.0; 5 days/week 86±12		Rose et al. (1976)
	for 7 weeks		
Mice			
ICR/Ha Swiss	ethanol:Tween 80: saline 135[a] (1:10:89)	27-28	Koshakji et al. (1984)
Hamsters			
Golden Syrian	olive oil 650[a]	73.5±22.8	Olson et al. (1980a)

[a]   Single dose.

Table 41. Elimination of TCDD in different species

Species/Strain	Route/Vehicle radioactivity	Dose[a] (µg/kg body weight)	Duration of study (days)	Half-life for elimination (days)	Eliminated (% of administered dose) Faeces	Urine	Reference for elimination study
Rats							
Sprague Dawley	oral/acetone: corn oil (1:9)	50	21	17.4 ±5.6	53.2	13.2	Piper et al. (1973)
Sprague Dawley	oral/corn oil	50	21	21.3 ±2.9	53.3	4.5	Allen et al. (1975)
Sprague Dawley	oral/acetone: corn oil (1:24)	1	22	31 ± 6	NR	ND	Rose et al. (1976)
Sprague Dawley	oral/acetone: corn oil (1:24)	1[b]	49	23.7	NR	3.1 ±0.2 (m) 12.5 ±5.1 (f)	Rose et al. (1976)
Sprague Dawley	ip/corn oil	400	7	NR	4.96 ±0.3	0.51 ±0.05	van Miller et al. (1976)
Mice							
C57BL/6	ip/olive oil	10	30	11	59.3	20.5	Gasiewicz et al. (1983b)
DBA/2	ip/olive oil	10	30	24.4	39.8	16.8	Gasiewicz et al. (1983b)
B6D2F$_1$	ip/olive oil	10	30	12.6	56.3	20.5	Gasiewicz et al. (1983b)
ICR/Ha Swiss	oral/ethanol: tween 80: saline (1:10:89)	135	11	20	78	4	Koshakji et al. (1984)
C57BL/6 Ah^b/Ah^d	ip/emulphor:	0.5	42	9.6	61.8	19.9	Birnbaum (1986)

```
                              ethanol:H2O
                              (1:1:18)
     C57BL/6 Ahd/Ahd   ip/emulphor:          0.5         42
9.6            55.9              26.8           Birnbaum (1986)
                              ethanol:H2O
                              (1:1:18)
     DBA/2 Ahb/Ahd    ip/emulphor:            0.5         42
10.8           71.5              13.6           Birnbaum (1986)
                              ethanol:H2O
                              (1:1:18)
```

Table 41 (contd).

Species/Strain	Route/Vehicle radioactivity	Dose[a] (µg/kg body weight)	Duration of study	Half-life for elimination (days)	Reference
life	Eliminated				
(% of administered dose)					
(days)	Faeces	Urine			

Mice (contd)

```
     DBA/2 Ahd/Ahd    ip/emulphor:            0.5         42
10.8           76.1              11.1           Birnbaum (1986)
                              ethanol:H2O
                              (1:1:18)
```

Guinea-pigs

```
     Hartley             ip/olive oil        2
23                 33                2            Gasiewicz &
                                                          Neal
(1979)
     NR             oral/NR            NR        22         22-
43         ND              ND          Nolan et al. (1979)
     Hartley        ip/olive oil       0.56      45       93.7 ± 15.5
26.2 ±3.6      3.1 ±1.2      Olson (1986)
```

Hamsters

```
     Golden Syrian      oral/olive oil      650        35         15.0
```

±2.5	NR	NR		Olson et al. (1980a)	
Golden Syrian	ip/olive oil	650	35	12.0 ±2.0 50.0±2.7	34.6±5.4 Olson et al. (1980a)

Monkeys

Rhesus (adult)	ip/corn oil	400	7	NR 3.75	1.06 van Miller et al. (1976)
Rhesus (infant)	ip/corn oil	400	7	NR 1.26	2 van Miller et al. (1976)

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[a]   Single dose, unless otherwise stated.
[b]   5 days/week for 7 weeks.
ND   = not detectable, NR = not reported, m = males, f = females, ip = intraperitoneal.

The authors concluded that the faecal content during the first 3 days represented mainly unabsorbed TCDD and that apparently more than 75% of the administered dose had been absorbed from the gastrointestinal tract. The radioactivity excreted daily through the urine ranged between 0.1 and 0.2% of the administered dose during the initial 12 days. Thereafter, the daily urinary radioactivity excretion increased from 0.25% of the administered dose to 0.43% by day 21. The total amount of ^{14}C excreted through the urine over a 21-day period was about 4.5% of the dose. On the basis of the daily faecal and urinary excretion, the authors calculated a half-time of 21.3 (± 2.9) days. In the rats killed on days 1, 3, 5, 7, 14, and 21 after administration, the total liver content was 56 (± 5), 54 (± 14), 54 (± 6), 54 (± 8), 45 (± 5), and 24 (± 4)% of the administered dose, respectively. At all intervals, the levels found in the liver exceeded those found in other organs. On days 5, 7, and 14 about 90% of the total radioactivity in the liver was present in the microsomal fraction.

Fries & Marrow (1975) fed Sprague Dawley male and female rats a diet containing 7 or 20 µg ^{14}C-TCDD/kg diet for 42 days. Thereafter all rats received the control diet for another 30 days. Two animals of each sex and TCDD dietary level were sacrificed at 14-day intervals. This treatment resulted in decreased food consumption, decreased

weight gain, and increased relative liver weight among both males and females. The concentration of TCDD-derived radioactivity in the liver, which was the principal tissue depot of both males and females, was directly proportional to the dietary intake of ^{14}C-TCDD. At the end of the 42-day feeding period, the ^{14}C levels in male livers indicated TCDD contents of 5.8 and 15.9 µg/kg of tissue for the lower and higher feed concentrations, respectively. The concentrations in the liver of female rats were similar. Analysis of the liver of rats killed 14 and 30 days after discontinuing TCDD exposure indicated a gradual decrease in TCDD liver concentration in both sexes. The steady-state body burden for TCDD was estimated to be 10-11 times the daily intake in both sexes. The whole-body and liver half-lives were calculated to be 12 and 11 days in males and 15 and 13 days, respectively, in females.

Rose et al. (1976) estimated the absorption of a single non-toxic dose of 1 µg ^{14}C-TCDD/kg body weight in male and female Sprague Dawley rats to be 84 ± 11% of the administered dose. The elimination of TCDD was followed for 22 days after administration and the faecal excretion accounted for most if not all of the elimination of TCDD and/or its metabolites. No radioactivity was detectable in urine and expired air. Twenty-two days after dosing, mean values of 1.26% and 1.25% of the dose/g liver and adipose tissue, respectively, were found. Much lower ^{14}C-activities were found in the thymus, kidney, and spleen, namely 0.09, 0.06, and 0.02% of the dose/g, respectively.

The whole-body half-life was estimated to be 31 days. Rose et al. (1976) also followed the fate of 5 daily doses per week of 0.01, 0.1, and 1.0 mg ^{14}C-TCDD/kg body weight given for 1, 3, and 7 weeks to male and female Sprague Dawley rats (Table 42).

According to Kociba et al. (1976), a dose level of 0.01 µg/kg per day, 5 days/week for 13 weeks, produced no overt toxic effects, whereas 0.1 or 1.0 µg/kg per day produced adverse effects, including some deaths in the high dose group. In the study by Rose et al. (1976), the dose level of 0.01 µg/kg per day resulted in no detectable ^{14}C, except in liver, fat, and excreta. Thus no kinetic calculations on a truly non-toxic dose could be performed. Pooling the results for all rats receiving 0.1 or 1.0 µg/kg per day, the absorbed dose corresponded to 86 ± 12% of the administered dose, with an individual variation of 66 to 93%. The overall rate constant for elimination corresponded to a half-time of 23.7 days, with an individual variation of 16-37 days. The radioactivity was eliminated primarily in the faeces, but the percentage of the dose excreted in the urine compared to that eliminated in the faeces tended to increase with time. At the

dose level of 1.0 µg/kg per day, males excreted in the urine 3.1 (± 0.2)% and females 12.5 (± 5.1)% of the cumulative dose over 7 weeks of exposure. The one female rat that died during the 7th week excreted 17.8% of the cumulative dose in the urine. Exhaled air was not examined in these studies. After 7 weeks of exposure, the average body burdens were 47.7 (± 8.8) and 37.1 (± 7.5)%, respectively, of the administered dose for the rats given 0.1 and 1.0 mg TCDD/kg per day. The main tissue depots of ^{14}C were the liver and adipose tissue. Radioactivity was also detected in thymus, kidney, and spleen at levels between 1 and 2% of that in liver. Direct chemical determination of liver samples confirmed the TCDD-concentrations calculated by radioactivity measurements. TCDD and/or its metabolites approached a steady-state body burden calculated to be 21.3 D_O for rats given a daily dose (D_O, µg/kg body weight) on 5 consecutive days per week for an infinite number of weeks, within 13 weeks over the dose range 0.01 to 1.0 µg/kg per day. It was thought unlikely that the dietary intake of extremely low levels of TCDD would result in the accumulation of toxic amounts in the rat.

The excretion and distribution of a toxic dose of 400 µg ^{3}H-TCDD/kg body weight was followed in male Sprague Dawley rats (Van Miller et al., 1976). Within 7 days, about 5.0% of the dose had been excreted in faeces and 0.5% in urine. The principal tissue depots for radioactivity were liver, muscle, and skin, which contained 43.0, 4.6, and 4.4% of the administered dose, respectively.

Table 42. Tissue distribution of TCDD-derived radioactivity in rats given oral doses of ^{14}C-TCDD[a]

	Tissue content of ^{14}C (µg equivalents of TCDD/kg tissue)[b]		
Tissue	1 week	3 weeks	7 weeks
Exposure level: 1.0 µg/kg body weight per day			
liver	49.5±3.6	110.2±37.1	204.0±52.2
adipose tissue	10.0±3.0	23.5±7.5	61.4±36.7
thymus	0.9±0.2	7.3±4.5	6.9±2.5

```
kidney                    0.9±0.2              1.9±0.9              5.5±5.1
spleen                    0.4±0.1              1.6±0.8              1.9±1.1
```

Exposure level: 0.1 µg/kg body weight per day

```
liver                     3.9±1.1             11.8±2.2             19.8±3.1
adipose tissue            0.9±0.6              2.7±0.8              4.5±0.7
thymus                    ND                   1.0±0.6              0.6±0.2
spleen                    ND                   0.6±0.4              0.3±0.1
kidney                    ND                   ND                   ND
```

Exposure level: 0.01 µg/kg body weight per day

```
liver                     ND                   0.8±0.1              1.6±0.5
adipose tissue            ND                   0.3 (2)c             0.3±0.1 (4)c
thymus                    ND                   0.6±0.1              ND
spleen                    ND                   0.6±0.3              ND
kidney                    ND                   ND                   ND
```

_____

[a]   From: Rose et al. (1976).

[b]   The mean ± standard deviation of 3 male and 3 female rats.

[c]   Indicates the number of animals with detectable levels of
       14_C-activity.

ND   = not detected.

     Kociba et al. (1976) gave Sprague Dawley rats TCDD in daily doses
of 0.01, 0.1, and 1 µg/kg body weight 5 days per week for 13 weeks by
gavage. The liver contained TCDD at a level of 324 (± 53) µg/kg wet
weight in males and 284 (± 21) µg/kg wet weight in females given
repeated TCDD doses of 1 µg/kg body weight per day. For the dose level
of 0.1 µg/kg body weight per day, the liver TCDD levels were 36 (± 4)
and 35 (± 4) µg/kg wet weight for males and females, respectively. A
dose of 0.01 µg/kg body weight per day resulted in TCDD liver levels
of 2.6 (0.6) µg/kg wet weight in males and 3.7 (± 0.4) µg/kg wet
weight in females.

     After feeding diets with TCDD levels corresponding to daily
dietary intakes of 0.001, 0.01, or 0.1 µg/kg body weight for 2 years,
the average concentrations of TCDD found in the liver of female
Sprague Dawley rats were 0.54, 5.1, and 24.0 µg/kg wet weight,
respectively (Kociba et al., 1978). The corresponding levels in
adipose tissue were 0.54, 1.7, and 8.1 µg/kg wet weight. A comparison
of liver TCDD levels found in rats given comparable daily doses of

TCDD for 13 weeks (Kociba et al., 1976) or 2 years (Kociba et al., 1978) indicates that with prolonged exposure the liver TCDD content reaches a plateau. This finding agrees well with the prediction obtained by mathematical analysis of the data resulting from experiments involving exposure of a few weeks (Rose et al., 1976).

The proportion of a single oral dose of ^{3}H-TCDD found in the liver of female Sprague Dawley rats was dependent both on the dose level and on the vehicle used (Poiger & Schlatter, 1980). Maximal retention occurred within 48 h after dosing. Increasing retention was observed up to a dose of 280 ng TCDD/rat. Hepatic retention 24 h after dosing was higher (36.7% of the dose) if TCDD was given in 50% ethanol than if it was given as an aqueous suspension of soil (37%, w/w), where it was 16-24.1% of the dose, or activated carbon (25%, w/w), where it was < 0.07% of the dose (see section 7.4).

Biliary excretion of radioactivity originating from 3H-labelled TCDD occurred at a more or less constant rate of 0.5 to 1% of the administered dose per day for 12 days following the administration of 100 µg TCDD/kg body weight in a female Sprague Dawley rat (Poiger & Buser, 1983). No severe toxic effects were observed within that time period.

McConnell et al. (1984) dosed female Sprague Dawley rats orally with either pure TCDD in corn oil or amounts of contaminated soil giving similar doses of TCDD. In general TCDD in soil was as potent an inducer of aryl hydrocarbon hydroxylase (AHH) as pure TCDD in corn oil. The hepatic concentration of TCDD was 40.8 µg/kg in the corn oil group, receiving 5 µg TCDD/kg body weight, and 20.3 µg/kg in the soil group, receiving 5.5 µg TCDD/kg body weight (see section 7.4).

Poiger & Schlatter (1980) studied the dermal absorption of ^{3}H-TCDD in hairless rats of the Naked ex Back-Cross and Holzman strain (200-250 g). Using the amount of TCDD-derived radioactivity found in the liver as an indicator of its absorption, they reported that the permeation of TCDD across the epidermis was highly dependent on the formulation used. The highest radioactivity in the liver, 14.8% of the administered dose, was detected when TCDD was applied as a methanolic solution. The hepatic recovery of the administered dose observed when TCDD was applied in polyethylene glycol 1500 with and without 15% water was 14.1 and 1.4%, respectively. Dermal application of TCDD in vaseline or adsorbed onto soil or activated carbon decreased the percentage of the dose recovered in the liver to 1.4, 1.7-2.2, and < 0.05%, respectively.

In studies by van den Berg et al. (1983), fly ash and crude or

purified toluene extracts of PCDD- and PCDF-containing fly ash from a
municipal incinerator were mixed with ordinary laboratory diet for
rats. Small portions (2 g) of these diets were fed to male Wistar rats
(300 g) every 24 h for 19 days, at which time the animals were
sacrificed. Tetra-, penta-, and hexa-chlorinated PCDDs and PCDFs in the
liver and adipose tissues of these rats were determined. Rats fed the
fly ash containing diet stored PCDDs and PCDFs in their livers at
concentrations that were at least 3 to 5 times lower than in the case
of rats fed comparable amounts of fly ash extracts (for the pentaCDD,
hexaCDF, and hexaCDD isomers, the concentrations were approximately
10-20 times lower). Generally PCDFs showed a higher retention in rat
liver than did the corresponding PCDDs. In the adipose tissue of rats
fed with fly ash extracts, retention was higher for penta- and
hexaCDDs than for the corresponding PCDFs.

In further fly ash studies, male Wistar rats (275 g) were fed for
up to 99 days a diet that included 2.5% HC1-pretreated fly ash
(containing PCDDs and PCDFs) from a municipal incinerator (van den
Berg et al., 1986a). A control group received standard diet. All
congeners retained in the liver of the rats had a 2,3,7,8-chlorine
substitution pattern. With the exception of 2,3,4,7,8-pentaCDF and
2,3,4,6,7,8-hexaCDF, liver retention for each congener was below 10%
of the group dose. The retention percentages of the various congeners
in the liver were almost equal at the time-points studied (34, 59, and
99 days), thus indicating a long half-life of these congeners in rat
liver.

Male Wistar rats fed 22.7 (± 1) µg or 120.7 (± 2.8) µg octaCDD
over a two-week period were found to retain about 1-2% of the given
dose in the liver (Williams et al., 1972). The heart, kidneys, spleen,
lung, skeletal muscle, testes, and urine contained no detectable
levels of octaCDD, but minor amounts were found in the adipose tissue

of the high-dose group. Faeces contained 61% and 37%, respectively, of
the low and high dose given. The presence of a large quantity of
octaCDD in the faeces compared to that in the bile 24-72 h after a
single oral dose of 58 mg octaCDD to bile-cannulated male Wistar rats
(400 g) indicated that the dioxin present in the faeces was mainly
unabsorbed octaCDD (Williams et al., 1972).

After 21 daily doses of 100 mg octaCDD containing 12.6 pg
[35]S-thio-heptaCDD to male Sprague Dawley rats, the radioactivity was
mainly recovered in the faeces and urine, the percentages of the
ingested radioactive dose being 93 (± 6) and 5.2 (± 0.8)% respectively
(Norback et al., 1975). The high faecal excretion suggests poor
absorption. Of the radio-active body burden, 50% was contained in the

liver. The microsomal fraction contained 96.3 (± 8.2)% of the hepatic radioactivity.

6.1.2 Studies on mice

In studies by Vinopal & Casida (1973), male white mice (20 g) were given tritium-labelled TCDD intraperitoneally in a single dose of 130 µg/kg body weight. Three days after TCDD administration to one mouse, 13% of the administered tritium was recovered in the faeces and 0.3% in the urine, while 32% was found in the liver and 0.3% in the kidneys. In another study, groups of two to six mice were killed at various time intervals after similar treatment with the same dose of ^{3}H-TCDD. One and 4 days after dosing, the liver contained about 15% of the dose, on the 8th day, 26%, on the 11th day, 22%, and on the 15th and 20th days, about 10%. The highest amount of ^{3}H-activity was found in the microsomal fraction of the liver. Somewhat lower activity was detected in the mitochondrial fraction and the nuclei. The supernatant fraction was practically devoid of any radioactivity. On day 8 when the highest levels were observed, the whole liver homogenate contained 26.7 (± 4.8)% of the administered dose, the microsomes 12.6 (± 3.8)%, the nuclei 7.8 (± 1.2)%, the mitochondria 6.2 (± 1.3)%, and the supernatant fraction 0.1 (± 0.0)% of the administered dose.

Coccia et al. (1981) described the effect of adding different substances to food on the persistence of TCDD in the liver of male C57Bl/6 mice. In one of the experiments, the test diet was given immediately after the administration of a single oral dose of 7.6 µg ^{3}H-TCDD/kg body weight. The hepatic radioactivity 14 days after dosing was 17.3, 6.3, 13.1, and 14.5% of the administered dose in animals fed standard chow containing 5% vegetable charcoal, 0.5% cholic acid, and 4% cholestyramine, respectively. When feeding of the test diet started 3 days after dosing, the hepatic retention of ^{3}H-TCDD was decreased to a similar extent.

Faecal and urinary excretion (Table 41), along with the formation of faecal, biliary, and urinary metabolites (see 7.2.1.1), of ^{3}H-TCDD was studied in male C57BL/6, DBA/2, and B6D2F1 mice after a single intraperitoneal dose of 10 µg ^{3}H-TCDD/kg body weight (Gasiewicz et al., 1983b). The principal tissue depot in C57BL/6 and B6D2F1 mice was the liver, followed by the adipose tissue. Most other tissues examined contained less than 1% of the administered dose. In DBA/2 mice, the adipose tissue contained more radioactivity than the liver. This difference may be due to the fact that these three strains of mice differ in their adipose tissue content, being 5.9, 11.5, and

5.0% of the body weight in C57BL/6, DBA/2, and B6D2F1 mice, respectively. The estimated half-lives of clearance of 3H-TCDD from the liver of C57BL/6, DBA/2, and B6D2F1 mice were 17, 27, and 13 days, respectively, and the corresponding figures for the half-life in the adipose tissue were 11, 42, and 11 days. The cumulative faecal elimination 30 days after dosing was 59.3, 39.8, and 56.3% of the administered dose in C57Bl/6, DBA/2, and B6D2F1 mice, respectively, and the corresponding figures for urinary elimination were 20.5, 16.8, and 20.5%.

Birnbaum (1986) studied in mice the distribution and excretion of a single intraperitoneal dose of 500 ng (45 µCi)3H-TCDD/kg body weight for up to 42 days after treatment. Two sets of congenic strains of mice were used, i.e., male C57Bl/6 and female DBA/2 mice, where within each congenic pair the mice differed only at the Ah locus (or at a limited number of genes closely linked to the Ah locus). The mice were bred and phenotyped by zoxazolamine paralysis time. The results, some of them summarized in Tables 47 and 48, suggested that, at the dose level studied, the distribution and excretion of TCDD were primarily governed by the total genetic background rather than by the allele present at the Ah locus.

When male ICR/Ha Swiss mice (27 - 35 g) were given a single oral dose of 135 µg ^{14}C-TCDD/kg body weight, about 71% and 1-2%, respectively, of the administered dose was eliminated via faeces and urine within the first 24 h (Koshakji et al., 1984). During the following 10 days, an additional 7% and 2% of the administered radioactivity were recovered in faeces and urine, respectively. Based on the estimated body burden of radioactivity, a whole-body half-life of 20 days was calculated.

The distribution of ^{3}H-TCDD in the skin of hairless (SKH:HR-1) mice after a single intraperitoneal dose of 6.3 µg TCDD/kg body weight was examined for up to 14 days (Puhvel et al., 1986). Most of the ^{3}H-TCDD in skin was localized in the dermis, although the concentration of ^{3}H-TCDD was consistently higher in the epidermis.

6.1.3 Studies on guinea-pigs

The retention of a single intraperitoneal dose of 2 µg ^{14}C-TCDD/kg body weight in various tissues of male Hartley guinea-pigs was determined 1, 3, 5, 7, 11, and 15 days after exposure (Table 43) (Gasiewicz & Neal, 1979). Three animals died and all animals lost 24 to 35% of the body weight during the study. The highest amount of radioactivity per tissue was found in the liver and

skin. The radioactivity in the liver increased with time, concomitant to the depletion of adipose tissues. Radioactivity in the skin decreased also with time. No signs of toxicity were seen when the cumulative excretion of a single i.p dose of 0.5 µg ^{3}H-TCDD/kg body weight in male Hartley guinea-pigs was studied (Gasiewicz & Neal, 1979). The faecal and urinary excretion of radioactivity was linear throughout the 23-day study. Approximately 1.4% of the administered dose was excreted daily during that period, and the faeces contained 94% of the excreted radioactivity.

The microsomal fraction of the liver in male Hartley guinea-pigs contained 40.7 to 47.4% of the hepatic radioactivity 1 day after a single ip dose of 0.3, 2.0, or 7.0 µg of ^{3}H- or ^{14}C-TCDD/kg body weight (Gasiewicz & Neal, 1979). Corresponding values for the crude nuclear fraction, the mitochondrial fraction, and the soluble fraction were 20.1-35.6%, 9.5-12.9%, and 7.6-26.4%, respectively. The subcellular distribution was similar 1 and 6 days after the low dose but, following the high dose, more radioactivity was present in the microsomal fraction and less was recovered in the crude nuclear and soluble fractions on day 6 after exposure.

Olson (1986) followed the distribution, elimination and metabolism (see section 6.2.1.1) of a single ip dose of 0.56 µg ^{3}H-TCDD/kg body weight in adult (335 to 625 g) male Hartley guinea-pigs for 45 days. One of seven animals died on day 27, but the remaining animals gained weight and exhibited no gross signs of toxicity. At termination the body composition was normal, and 61% of the administered radioactivity was recovered in the twelve investigated tissues at the end of the study. The adipose tissue contained 36% of the dose; liver, pelt, and skeletal muscle plus carcass contained each 7% of the dose; the gastrointestinal tract contained about 2% of the dose, and remaining tissues contained less than 0.5% of the dose. Urinary and faecal elimination followed apparent first-order kinetics, with half-lives of 82.5 (± 22.4) and 94.4 (± 14.7) days, respectively.

Table 43. Tissue content of TCDD-derived ^{14}C (% of dose/g tissue)[a] in guinea-pigs following a single
dose of ^{14}C-TCDD[d]

Days

after exposure

Tissue	1	3	5	7	11	15
Perirenal adipose	3.2±1.0	4.1±0.4	2.1±0.4	1.3±0.2	2.1±0.2	
Epididymal adipose	1.5±0.8	3.8±0.5	3.4±0.7	3.2±0.1	3.9[b]	2.5±1.1
Adrenal	1.4±0.3	1.4±0.2	0.9±0.1	1.2±0.3	2.1±0.9	1.7±0.2
Liver	1.1±0.4	1.5±0.4	1.3±0.2	1.1±0.2	2.2±0.2	3.2±0.3
Liver[c]	11.4±3.3	15.5±3.3	14.0±2.3	12.0±1.9	21.2±2.3	29.6±2.7
Spleen	0.7±0.3	0.5±0.3	0.2±0.1	0.4±0.2	0.4±0.2	0.5±0.1
Duodenum	0.4±0.2	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.3±0.1
Pancreas	–	–	0.2±0.1	0.5±0.3	0.4±0.3	0.3±0.1
Stomach	0.2±0.1	0.3±0.1	0.1±0.1	0.2±0.1	0.3±0.1	0.3±0.1
Testes	0.2±0.1	0.3±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.2±0.1
Kidneys	0.3±0.1	0.3±0.1	0.2±0.1	0.4±0.1	0.8±0.4	0.7±0.1
Bone marrow	0.3±0.1	0.5±0.1	0.2±0.1	0.4±0.1	0.4[b]	0.2±0.1
Lungs	0.3±0.1	0.2±0.1	0.2±0.1	0.4±0.1	0.5±0.2	0.6±0.1
Skin[c]	13.8±0.7	16.3±0.3	15.8±2.4	6.5±0.8	6.5±0.7	6.7±0.6
Brain, heart, skeletal muscle	<0.25					

[a]   Mean ± standard error for three animals, unless indicated otherwise.

[b]   Mean of two animals.

[c]   Percentage of dose/tissue.

[d]   From: Gasiewicz & Neal (1979).

6.1.4 Studies on hamsters

     In studies by Olson et al. (1980a), Golden Syrian hamsters
absorbed about 73.5% of a single oral dose of 650 µg ^{3}H-TCDD/kg body
weight, a dose that produced thymic atrophy and body weight loss in
several of the animals. The distribution of radioactivity in various
tissues 1, 3, 10, and 20 days after administration is given in Table
44. The principal depots were the liver and adipose tissue. A similar
pattern of distribution was obtained when the same dose was given
intraperitoneally. The elimination of radioactivity in faeces and
urine was followed for 35 days after a single intraperitoneal (ip) or
oral dose of 650 µg/kg body weight (Olson et al., 1980a). The
half-life for elimination was 12.0 (± 2.0) days and 15.0 (± 2.5) days
for the ip and oral routes, respectively. Of the excreted
radioactivity 41% occurred in urine and 59% in faeces.

     The hepatic retention of PCDDs and PCDFs from dietary intake of
HC1-pretreated fly ash from a municipal incinerator was studied in
male Golden syrian hamsters (van den Berg et al., 1986b). The livers
were analysed for tetra-, penta-, and hexaCDDs and PCDFs after feeding
the diet, which contained 25% fly ash, for 34, 58, and 95 days. No
detectable hepatic retention was observed after 34 days. The highest
retention after 95 days was 8.4% for 2,3,4,7,8-pentaCDF, but the
retention was generally below 5% of the total dose. With the exception
of 2,3,4,6,7-pentaCDF, only 2,3,7,8-substituted PCDDs and PCDFs were
retained. Constant relative concentrations were found for the
2,3,7,8-substituted PCDDs and PCDFs at the time points studied.

6.1.5 Studies on monkeys

     Van Miller et al. (1976) gave three adult female rhesus monkeys
and four male infant rhesus monkeys a single intraperitoneal dose of
400 µg 3H-TCDD/kg body weight in corn oil. This dose resulted in a
loss of body weight: 10.8% for adults and 20.7% for infants, and light
microscopic changes in the liver. Over a 7-day period the adult
monkeys excreted 1.06% of the dose in the urine and 3.75% in the
faeces. During the same period the infant monkeys excreted
approximately 2% of the administered dose in urine and about 1.26% in
the faeces. The authors questioned the accuracy of these figures owing
to the difficulty of separating the two types of excreta from infant
monkeys. The total tissue concentrations of radioactivity 7 days after
dosing are given in Table 45. The principal tissue depots of
radioactivity in adult monkeys were adipose tissue, skin, liver, and
muscle; they contained 16.2, 13.1, 10.4, and 8.6% of the administered

dose, respectively. The distribution in infant monkeys was 35.6% in
muscle, 22.7% in skin, and only 4.5% of the dose in the liver.

Table 44. Tissue distribution of TCDD-derived radioactivity in Golden
    Syrian hamsters at 1, 3, 10, and 20 days following a single oral
    dose of 650 µg ^{3}H-TCDD/kg body weight[a]

Tissue content of ^{3}H (% of dose/g tissue)[b]

Tissue	Day 1	Day 3	Day 10	Day 20
Liver	4.03±1.00	5.32±.82	3.19±.93	0.86±0.09
Liver[b]	12.74±3.21	20.44±3.45	9.69±0.99	3.70±0.29
Perirenal adipose	2.93±0.87	3.48±0.56	1.38±0.28	0.32±0.03
Adrenals	1.56±0.52	1.12±0.14	0.47±0.08	0.10±0.01
Pancreas	0.39±0.20	0.61±0.13	0.62±0.26	0.21±0.04
Kidneys	0.60±0.16	0.64±0.11	0.60±0.32	0.12±0.03
Spleen	0.30±0.08	0.24±0.05	0.43±0.26	0.07±0.02
Thymus	0.49±0.14	0.34±0.11	–	0.05±0.02
Skin	0.84±0.26	0.31±0.07	0.56±0.18	0.03±0.01
Stomach	0.34±0.07	0.55±0.09	0.65±0.39	0.16±0.06
Duodenum	0.51±0.13	0.47±0.09	0.55±0.28	0.07±0.02
Jejunum	0.59±0.15	0.71±0.20	0.39±0.16	0.08±0.02
Ileum	0.41±0.12	0.35±0.05	0.37±0.21	0.06±0.02
Colon	0.92±0.27	0.60±0.14	0.34±0.07	0.06±0.01
Caecum	0.39±0.11	0.41±0.10	0.28±0.12	0.05±0.01
Lungs	0.38±0.09	0.37±0.05	0.41±0.25	0.07±0.03
Skeletal muscle	0.20±0.07	0.15±0.05	0.15±0.03	0.04±0.02
Heart	0.14±0.03	0.13±0.02	0.15±0.08	0.03±0.01
Testes	0.10±0.04	0.32±0.13	0.13±0.04	0.03±0.01
Blood	0.12±0.02	0.14±0.03	0.12±0.06	0.02±0.01
Brain	0.03±0.01	0.05±0.01	0.06±0.02	0.01

[a]   From: Olson et al. (1980a).

[b]   All values are the mean (± standard error) of four hamsters.

c   Percentage of dose/liver.

Table 45. Tissue distribution of TCDD-derived radioactivity in adult
      and infant rhesus monkeys 7 days following a single intraperitoneal
      dose of 400 µg ^{3}H-TCDD/kg body weight[a]

	(% of dose/tissue) Tissue content of 3H[b]	
Tissue	Adult	Infant
Liver	10.4±6.9	4.51±1.60
Brain	0.58±0.34	1.41±1.40
Spleen	0.028±0.013	0.026±0.004
Small intestine	0.87±0.39	1.47±0.64
Large intestine	1.29±0.12	0.64±0.24
Muscle[c]	8.62±2.39	35.6±14.4
Skin	13.1±4.9	22.7±8.8
Adipose tissue[d]	16.2±5.8	

a   Fom: Van Miller et al. (1976).
b   Mean (± standard deviation) of three adults or four infants.
c   Total muscle was taken as 40% of body weight.
d   Quantities in infant monkey were insignificant. For adult
     monkeys, the calculation was based on an estimate of 300 g
     mesenteric fat.

     The concentration of TCDD in samples of faeces, urine, and fat
were measured by GC-MS at intervals after dosing up to 715 days in an
adult female rhesus monkey (Macaca mulatta) given a single oral
dose of 1 µg TCDD/kg body weight (McNulty et al., 1982b). During the
3 months after dosing the monkey lost 50% of its body weight but then
began to gain weight again. The level of TCDD in faeces was high for
4 days and then fell to very low or undetectable levels. TCDD in urine
was very low at all time points. The apparent half-life of TCDD in
adipose tissue was about 1 year.

     There were no significant time-dependent changes in TCDD-derived
radioactivity found in the tissues investigated from marmosets during

the 3-week-period following subcutaneous treatment with 5 µg ^{14}C-TCDD/kg body weight, except for a minor decrease in levels found in the adipose tissue (Krowke, 1986). The data thus indicate a long half-life for TCDD in marmosets.

6.1.6 Studies on dogs

A one-year-old male beagle dog received a total dose of 5.4 µg of TCDD enterally by direct introduction into the duodenal lumen in four portions of 1-2 µg, with intervals of 2-7 days between treatments (Poiger et al., 1982). Severe toxic symtoms preceeded the death of the animal 17 days after the first dose. The excretion of radioactivity in the bile reached a maximum on day 1 or day 2 following administration. Significantly more biliary radioactivity was found after administration of doses 3 and 4 than after the administration of doses 1 and 2, suggesting that TCDD-administration stimulated its own metabolism. It was later demonstrated in a 18-months-old male boxer that TCDD-pretreatment (10 µg/kg body weight) stimulated the biliary excretion of ^{3}H-labelled TCDD (32.8 ng/kg body weight), whereas phenobarbital-pretreatment had no effect when compared to the biliary excretion without pretreatment (Poiger & Schlatter, 1985). All the experiments were carried out in the same animal, which had enough time between treatments for the radioactivity to return almost to the background level.

6.1.7 Studies on cows

The major routes for elimination of ^{3}H-TCDD in Holstein cows (500-650 kg) after oral doses of 0.05µg (two cows) or 7.5µg (one cow) TCDD/kg body weight were faeces > milk > urine (Jones et al., 1987). Fifty percent of the administered dose was eliminated in faeces, the major part in the first few days after treatment. Three lactating Holstein cows received commercial technical grade pentachlorophenol orally by gelatine capsule at a dose rate of 10 mg/kg body weight twice daily for 10 days and once daily for the following 60 days (Firestone et al., 1979). One cow served as a control and received gelatine capsules containing only ground corn. The pentachlorophenol composite used contained ten PCDD congeners (0.1 to 690 mg/kg) and eight PCDF congeners (0.9 to 130 mg/kg). Faeces collected on day 28 of the treatment period contained three hexaCDDs (0.05 to 0.63 µg/kg), two heptaCDDs (21.3 to 33.1 µg/kg), and octaCDD (290 to 429 µg/kg). Faeces also contained hexa-, hepta-, and octaCDF. Milk, body fat, and blood contained only three of the PCDD congeners present in the pentachlorophenol composite, namely 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD. Milk samples also contained hexa-, hepta-, and octaCDF. The average concentrations of

1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, octaCDD, and octaCDF in the composite milk fat at the end of the treatment period were 20, 40, 25, and 2 mg/kg respectively. Similar concentrations were found in body (shoulder) fat at the end of the treatment period (13, 24, and 32 mg/kg, respectively, of 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD). Levels of dioxins in the blood were approximately 1000 times below the values in milk or body fat. The average daily excretion of 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD in the milk during days 40-70 was about 20, 40, and 23 mg, corresponding to 33, 3, and 0.6% of the daily intake of PCDDs. One

hundred days after the cessation of treatment, the average values for 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD in shoulder fat and milk fat were 2.5, 6.6, 5.6 mg/kg and 4.3, 6.9, 3.0 mg/kg, respectively.

6.1.8 In vitro studies

The uptake of ^{3}H-TCDD in human fibroblasts was less efficient when the TCDD was associated with the high density lipoprotein (HDL) than with the low density lipoprotein (LDL), and even less when associated with serum (Shireman & Wei, 1986). From studies in mutant human fibroblasts lacking the normal LDL cellular receptor, the authors concluded that the LDL receptor pathway was involved in the cellular uptake of TCDD. The uptake from LDL was time, temperature, and concentration dependent.

6.2 Metabolic Transformation

6.2.1 Studies on mammals

6.2.1.1 In vivo studies

The possible existence of a urinary metabolite of TCDD was first suggested by the finding of Allen et al. (1975) that the radioactivity in urine of 14C-TCDD treated rats was highest from week 2 to 3. Later both Poiger & Schlatter (1979) and Ramsey et al. (1982) presented evidence for the in vivo biotransformation of TCDD in the rat. Male Sprague Dawley rats were given two, four, or six daily oral doses of approximately 15 µg ^{14}C-TCDD/kg body weight, and the bile was collected for 24 h following the last dose (Ramsey et al., 1982). Using high pressure liquid chromatography, at least eight metabolites of TCDD were found in the bile from these rats. Incubation of the bile with ß-glucuronidase indicated the presence of glucuronide conjugates among the metabolites. Poiger & Schlatter (1979) incubated similarly the bile or the dialysate with glucuronidase/arylsulfatase, and the

dichloromethane-extractable radioactivity increased from 1.5% to 75%. Their results indicate the elimination of TCDD-metabolites in the form of water-soluble sulfate and glucuronide conjugates. ^{3}H-TCDD metabolites extracted from the bile of one-year-old beagle dogs with ethanol (Poiger et al., 1982) were given to bile-duct-cannulated female Sprague Dawley rats (250 g) as single oral doses of 7.8-20.8 μg ^{3}H-TCDD-metabolites/kg body weight (Weber et al., 1982b). The mean 24-h elimination of radioactivity was 86.7 (± 6.7)% of the dose, 8.2% occurring in urine, 31.3% in bile, and 46.9% in faeces. A delay in the excretion of radioactivity in rats whose bile ducts were not cannulated suggested an enterohepatic circulation in the rat of the ^{3}H-TCDD-metabolites from the dog. The radioactive material in the rat bile seemed to be conjugated forms of the metabolites from the dogs. A metabolic breakdown scheme of TCDD in the rat and dog (Fig. 3) was proposed by Poiger & Buser (1983). The major metabolite seems to be formed via cleavage of an ether bond.

The metabolic fate of a single ip dose of 10 μg, 500 μCi TCDD/kg body weight in male C57Bl/6, DBA/2, and B6D2F1 mice was studied by Gasiewicz et al. (1983b). Samples of urine, bile, and faeces, collected on days 5 to 8, 8, and 7 after treatment, respectively, were extracted and analyzed for metabolites of TCDD by HPLC. Unmetabolized TCDD was detected in faeces but not in urine or in bile. More than 85% of the total radioactivity eliminated was present as metabolites of TCDD in all three mouse strains. Metabolites in the bile appear to be less polar than in urine. Qualitatively the elution profiles for urine, bile, and faeces from all three strains appeared to be quite similar.

A dose of ^{3}H-TCDD (0.56 μg/kg body weight) that produced no gross toxicity was given ip in olive oil to six adult male Hartley guinea-pigs (Olson, 1986). Metabolites of TCDD were found in organic extracts of the liver, kidney, perirenal adipose tissue, and skeletal muscle in amounts corresponding to 13, 4, 8, and 28%, respectively, of the recovered radioactivity in these organs 45 days after dosing. These figures suggest that TCDD-metabolites are not efficiently eliminated from tissues in guinea-pigs. All radioactivity in urine and bile represented metabolites of TCDD, whereas in faeces most (70-90%) contained unchanged TCDD. Of the radioactivity administered, 73.4% was eliminated as unchanged TCDD in faeces and 25.7% as metabolites of TCDD in urine and faeces. The presence of TCDD in faeces and its absence in bile suggest that direct elimination of TCDD from the blood to the intestinal lumen may occur. The HPLC elution profiles for metabolites were similar, although not identical, for bile, urine, and tissues. Taken together, the data by Olson (1986) indicate that metabolism does not appear to have a major role in the ultimate

elimination of TCDD in the guinea-pig.

Olson et al. (1980a) collected bile and urine from Golden Syrian hamsters that had been treated with a single ip dose of 650 µg ^{14}C-TCDD/kg body weight 7 days earlier. By means of HPLC, one major and several minor metabolites of ^{14}C-TCDD were demonstrated both in bile and urine. No metabolites of ^{14}C-TCDD were detectable in liver and adipose tissue, thus suggesting a rapid clearance of biotransformed products of TCDD.

A one-year-old beagle dog was cholecystectomized and a Thomas cannula was implanted about 3 months before the first dose of TCDD (Poiger et al., 1982, Poiger & Buser, 1983). A total dose of 5.4 mg was administered enterally in four portions of 1.8, 1.08, 1.08, and 1.44 mg on days 0, 2, 7, and 13. Five phenolic metabolites of TCDD excreted in dog bile were identified by combined gas chromatography-mass spectrometry. Severe toxic symptoms preceded the death of the dog 17 days after the first dose. A metabolic breakdown scheme of TCDD in the dog (Fig. 3) was proposed by Poiger & Buser (1983), lateral hydroxylation of TCDD seeming to be the major route of metabolism.
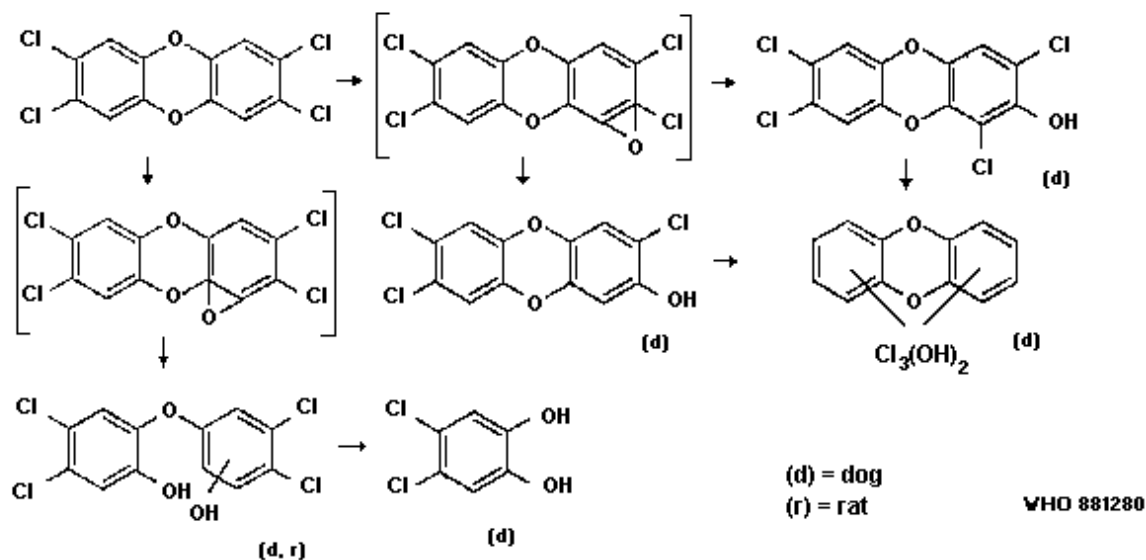

Fig. 3. Proposed metabolic breakdown scheme for TCDD. From: Poiger & Buser (1983).

6.2.1.2 In vitro studies

     Although there is evidence of different metabolites of TCDD and
the possibility of its metabolic transformation was suggested as early
as 1975 (Allen et al., 1975; Rose et al., 1976), it was only in 1982
that specific metabolites were identified by Sawahata et al. (1982).
They incubated TCDD with isolated rat hepatocytes at 37 ©C for 8 h,
and the resulting incubation mixture was subjected to HPLC. The major
peak of radioactivity not corresponding to TCDD was incubated with
ß-glucuronidase in order to split the possible glucuronide
conjugate(s) of TCDD or its metabolite(s). They found that
4,5-dichlorocatechol and 4,5-dichloroguaiacol are potential
metabolites of TCDD, but due to the limited amount of material the
identity of these metabolites was not confirmed by gas
chromatography-mass spectrometry. Two other metabolites of TCDD,
namely 1-hydroxy-2,3,7,8-tetrachloro-dibenzo-p-dioxin and
8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin, were isolated by means of
HPLC, and were identified by mass spectrometry.

     Primary hepatocytes from Sprague Dawley rats and Hartley
guinea-pigs have been used to study the metabolism of 14C-TCDD
(Wroblewski & Olson, 1985). The overall metabolism was 2.8 times
greater in rats than in guinea-pigs. The metabolism of 14C-TCDD was
increased 3.2-fold in rats pretreated with TCDD (5 µg TCDD/kg body
weight ip 72 h prior to isolation of hepatocytes), but no effect was
found in similarly TCDD-pretreated guinea-pigs, or in phenobarbital-
pretreated rats (80 mg/kg body weight ip for 3 days, beginning 4 days
prior to isolation of hepatocytes). Hepatocytes from TCDD-pretreated
rats metabolized TCDD 9 times more rapidly than similarly pretreated
guinea-pig hepatocytes. TCDD may be metabolized by an inducible form
of cytochrome P448 which is expressed in rats but not guinea-pigs.
These differences in metabolism may play a major role in explaining
the differences in species susceptibility to the acute effects of
TCDD.

6.3 Transfer Via Placenta and/or Milk

     The transplacental passage of ^{14}C-TCDD has been studied by
Khera & Ruddick (1973). Pregnant Wistar rats were given ^{14}C-TCDD in
a single oral dose of 200 µg/kg body weight on gestation days 16, 17,
or 18 and were killed 6 h after dosing. ^{14}C-activity was detected in
maternal tissues and also in the fetuses and the placenta. Assuming
that all the ^{14}C-activity found in the samples was present as
[14]C-TCDD, the following levels (ng/gram tissue) were found for

gestation days 16, 17, and 18 respectively: maternal liver 339 (± 15), 339 (± 19), and 275 (± 20); maternal blood 25 (± 11), 19 (± 9), and 10 (± 3); placenta 25 (± 6), 38 (± 4), and 41 (± 3); and fetus 11 (± 3), 15 (± 1), and 16 (± 1). Studies by Moore et al. (1973) indicated that

the passage of TCDD or its metabolites into milk could be of importance, as TCDD-related effects were observed in sucklings, nourished by lactating mothers given, after delivery, a single oral dose of 1 or 3 µg/kg body weight.

The TCDD concentration in livers of pregnant NMRI mice, at a given dose, was significantly lower than in livers of non-pregnant mice (Krowke 1986). The concentration of TCDD in the liver of non-pregnant mice was about 5 times higher than in pregnant mice 7 days after a s.c. dose.

Nau & Bass (1981) studied the transfer of ^{14}C-TCDD to embryos and fetuses in NMRI mice. The animals were given a single dose of 5, 12.5, or 25 µg TCDD/kg body weight by gavage or by s.c. or ip injection at day 16 of gestation to study the transfer of TCDD to the fetus. The animals were killed two days later and various tissues were analyzed for radioactivity. No evidence was found to indicate a major first pass effect following oral administration. Maternal livers contained the highest levels of TCDD, 4.1 to 10.5% of the radioactive dose administered, which was about one order of magnitude higher than in extrahepatic maternal tissues, including placenta. Fetal liver and extrahepatic tissues contained low levels of radioactivity corresponding to 0.09 to 1.41% and 0.05 to 0.14%, respectively, of the dose administered to the dams. More radioactivity was recovered in the placenta and fetus when TCDD was given either as a single ip dose of 25 µg/kg body weight on day 10 of gestation or as 5 daily ip doses of 5 µg/kg body weight on days 7 to 11, when compared to a single i.p. dose of 25 µg/kg body weight on gestation day 7. Oral dosing of 30 µg ^{14}C-TCDD (0.332 µCi/ µg)/kg body weight to pregnant C57Bl/6 mice on gestation day 11 resulted in 0 to 14% embryomortality on gestation days 12 to 14 (Weber & Birnbaum, 1985). About 0.03% of the radioactive dose was contained in the embryo and in the placenta on days 12 to 14 of gestation. The maternal liver contained 67.4, 67.9, and 50.6% of the administered radioactive dose on gestation days 12, 13, and 14, during which days the cumulative elimination of radioactivity in urine and faeces was 2.4 and 53.3% of the dose, respectively.

The transfer of ^{14}C-TCDD via placenta and milk and the distribution of the transformed TCDD between various embryonic and fetal tissues were studied in NMRI mice (Nau et al., 1986). Dams were given a single dose of 25 µg ^{14}C-TCDD (45 mCi/mmol)/kg body weight

either orally, subcutaneously, or intraperitoneally. To differentiate between postnatal and in _utero_ exposure, the experimental design included cross fostering. Depending on the route of administration, from 0.02 to 0.07% of the administered radioactivity was found in the liver of the fetus at birth. The highest levels were noted after ip administration and the lowest after oral intubation. The corresponding values one week after birth were 0.05 to 0.20%. The hepatic radioactivity in the neonate reached a peak 1 week after birth and then decreased slowly throughout and after lactation. The levels of

TCDD-derived radioactivity in extra-hepatic tissues of the offspring were approximately one order of magnitude lower than the hepatic levels. Very little radioactivity was found in the stomach filled with milk, indicating that TCDD ingested from milk was rapidly absorbed in the stomach.

Arstila et al. (1981) studied the excretion of TCDD in goat milk after a subchronic administration of 200 ng TCDD per day for 2 months in the first experiment and of 400 ng TCDD per day for one month in the second experiment. The minimal detectable concentration in this study was declared to be below 5 ng/litre. The maximum concentration of TCDD in milk in the first experiment was 20.8 (± 6.6) ng/litre and in the second experiment 19.3 (± 6.6) ng/litre. After 18 weeks feeding with TCDD the levels had dropped to 4.2 and 3.6 ng/litre, respectively.

The secretion of TCDD in milk and cream has also been studied in lactating dairy cows kept on a diet containing 10, 30, 100, 300, or 1000 mg/litre 2,4,5-T, corresponding to TCDD levels of 5, 15, 50, 150, and 500 ng/litre (Jensen & Hummel, 1982). This resulted in levels of TCDD in the excreted milk of below detection limit, 3, 10, 16-22, and 42-89 ng/litre, respectively, indicating that about 10-20% of the dose given was eliminated in the milk. The levels in cream were about ten times higher than those in milk.

One lactating Holstein cow, receiving commercial technical-grade pentachlorophenol containing several PCDDs and PCDFs orally in gelatine capsules at a dose rate of 10 mg/kg body weight twice daily for 10 days and once daily for the following 60 days, calved 151 days after treatment was stopped (Firestone et al., 1979). The PCDD content of blood, body fat (shoulder from the cow and hind quarter of the calf), and milk fat was determined 14 days later. The detected congeners were 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD and their levels were 12, 13, 20 ng/litre and 27, 14, 6 ng/litre in the blood of the cow and calf, respectively. Corresponding values for body fat were 4.8, 11.1, and 6.1 µg/kg in the cow and 2.3,

1.9, and 0.5 µg/kg in the calf. The milk fat from the cow contained
2.2, 4.4, and 3.3 µg/kg of the respective congeners.

6.4 Matrix Effects on the Uptake ("Bio-availability")

     Uptake of TCDD and other PCDD congeners is highly dependent upon
the formulation in which it is applied. Although conflicting results
have been obtained from studies where TCDD in soil was administered
(McConnell et al., 1984; Umbreit et al., 1985, 1986a, 1986b), most
available data support the idea that mixing TCDD with soil or
activated carbon results in the adsorption of TCDD to the soil
particles, thus reducing the availability of TCDD. Contact time of
TCDD with soil seem to influence the availability, probably because
the binding of TCDD to soil particles becomes strengthened (Poiger &

Schlatter, 1980). Studies elucidating the matrix effects of various
soils or activated carbon on the TCDD-responses in several animal
species are summarized in Table 46.

     Poiger & Schlatter (1980) showed decreasing hepatic recovery of
TCDD in rats 1 day after dosing using suspensions of ethanol, soil, or
activated carbon as vehicles. About 50% lower hepatic retention of
TCDD was obtained in the rat, 6 days after dosing, when Minker stout
site soil was the vehicle as compared to corn oil (Lucier et al.,
1986). However, in this study hepatic enzyme-induction (AHH and UDPGT)
was similar in the two vehicle groups.

     The bioavailability of TCDD from environmentally contaminated
soil samples has been studied in young guinea-pigs after intragastric
administration (McConnell et al., 1984). Groups of six animals each
were given soil samples corresponding to doses of approximately 1, 3,
or 10 µg TCDD per kg body weight. The doses were based on analyses of
soil siftings (60-gauge mesh) from the Times Beach and Minker Stout
sites, which indicated concentrations of 770 and 880 µg TCDD/kg,
respectively. Controls received soil samples in which no TCDD, PCBs,
or PCDFs were detected. For comparison, pure TCDD in corn oil was
given at either 0, 1, or 3 µg/kg. The observation time was 30 days.
LD_{50} values calculated in this study were 1.75 µg/kg for TCDD in
corn oil, 7.15 µg/kg for Times Beach soil, and 5.50 µg/kg for the
Minker Stout site soil. An exact percentage for bioavailability was
not calculated in this study, but the TCDD content of the livers of
exposed guinea-pigs indicated a highly efficient absorption of TCDD
from soil.

     TCDD in contaminated soil from a 2,4,5-T manufacturing plant and
from a metal salvage yard (Newark, New Jersey, USA) had a low

bioavailability (0.5% and 21.3%, respectively) in guinea-pigs (Umbreit et al., 1985, 1986a). The soils were given as single oral doses in a 10% aqueous suspension in 5% gum acacia. The bioavailability was judged by the hepatic concentration of TCDD 60 days after dosing in guinea-pigs receiving site soils, decontaminated soil, and TCDD-recontaminated soil.

The difference in bioavailability of TCDD from 2,4,5-T-manufacturing site soil in Newark (New Jersey) and from Times Beach Site soil (Missouri) when given orally to guinea-pigs was confirmed by Umbreit et al. (1986b).

Bonaccorsi et al. (1984) gave to rabbits seven daily doses of TCDD in corn oil, TCDD-contaminated Seveso soil, or recontaminated soil. Taking the hepatic recovery of TCDD in the corn oil group as 100% "bioavailability", the decrease in "bioavailability" of TCDD from Seveso soil was 68%. The decrease in "bioavailability" of recontaminated soil varied from 0 to 44% with the doses used.

Table 46. Matrix effects of various soils on TCDD-responses, as related to the estimated bioavailability of TCDD given orally to different species.

Species/ Lethality Length of study (days)	Vehicle/matrix Hepatic recovery of TCDD[e] "bio-availability"	Dose of Estimated TCDD[c]	Reference
Rat 1 day	50% ethanol 36.7[f] recontaminated soil[a] 24.1[f] recontaminated soil[b] 16.0f activated carbon	14.7 ng 12.7, 22.9 ng 21.2, 22.7 ng 14.7 ng	Poigner & Schlatter (1980) < 0.07[f]
Rat	corn oil	1.0, 5.0	7.6

```
and 40.8                            Lucier et al.
    6 days      Minker Stout site soil  1.1, 5.5                        1.8
and 20.3      50%                  (1986)


    Guinea-     corn oil                  1, 3            1/6, 6/6
1.6, 13.3                             McConnell et
    pig         Times Beach site soil   1.3, 3.8, 12.8  0/6, 1/5, 5/5     <
1.0-34.3        Efficient          al. (1984)
    30 days     Minker Stout site soil  1.1, 3.3, 11.0  0/6, 2/6, 6/6     <
1.0-25.7        absorption from
                recontaminated soil      10.0
6/6               45.4                soil (85%)


    Guinea-     corn oil
6               5/8                                            Umbreit et al.
    pig         2,4,5-T-manufacturing   3, 6, 12        0/8, 0/7, 0/7    0.09
(high dose)  0.5%                (1985, 1986a)
    60 days     site soil (Newark,
                New Jersey, USA).
                Metal salvage yard soil
0.32                              0.23            21.3%
                (Newark)
                recontaminated soil      6              6/7               18
```

Table 46 (contd).

Species/	Vehicle/matrix	Dose of		Reference
Lethality	Hepatic	Estimated		
Length		TCDD[c]		
recovery of	"bio-			
of				
study				
TCDD[e]	availability"			
(days)				

```
    Guinea-     2,4,5-T-Manufacturing   3, 5, 10        0/18,
1/20, 1/18                            Umbreit et al.
    pig         site
soil,
(Newark)                                                      (1986b)
    60 days     Times Beach site soil   1, 3, 10        2/19, 2/20, 8/14
                recontaminated soil      6              19/20


    Rabbit      corn oil                20, 40, 80[d]
```

```
0.26-2.7                                    Bonaccorsi et
    7 days        Seveso soil              80, 160d
0.88-2.2            > 32%              al. (1984)
                 recontaminated soil     20, 40, 80d
0.26-1.5            > 66-100%
```

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a   Contact time = 10-15 h.

b   Contact time = 8 days.

c   µg/kg body weight, unless otherwise stated.

d   ng/kg body weight per day.

e   µg/kg liver, unless otherwise stated.

f   % of dose.

Considerably lower hepatic levels of PCDDs and PCDFs were observed in rats fed a diet containing fly ash from municipal incinerators compared with those fed a diet containing extracts from the same fly ash (van den Berg et al., 1983).

Dietary intake of soot-containing TCDD produced 60% mortality in male and female guinea-pigs on days 46 and 60, respectively, at which time the estimated TCDD-consumption was 1.3 µg/kg body weight for males and 1.9 µg/kg body weight for females (DeCaprio et al., 1983, see also section 8.2.3). These data thus suggest a high uptake of TCDD from the soot matrix.

7.   EFFECTS OF TCDD AND OTHER PCDDs ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

7.1 Acute Toxicity

7.1.1 In vivo studies on mammals

The range of doses required to cause death varies considerably between species, as well as between strains of species, and with sex, age, and route of administration within a single strain (Table 47). More than an 8000-fold difference exists between the dose of TCDD reported to cause 50% lethality to male Hartley guinea-pigs, the most sensitive species tested (Schwetz et al., 1973), and the corresponding dose for male Golden Syrian hamsters (Henck et al., 1981). The rat seems to be the second most sensitive species, although there is a

more than 200-fold variability in LD_{50} values between different strains. The oral LD_{50} value was 22 µg TCDD/kg body weight for male Sherman rats (Schwetz et al., 1973), whereas Walden & Schiller (1985) found LD_{50} values ranging from 164 to 340 µg TCDD/kg body weight when male Fisher 334 N rats from three different suppliers were tested. The Han/ Wistar-strain of rat has been demonstrated to be particularly resistant to TCDD-exposure (Pohjanvirta & Tuomisto, 1986). Among the five rats per dose group (0, 1500, 2000, 2500, or 3000 mg TCDD/kg body weight) only one animal died within the 39-40 days observation period.

Monkeys (McConnell et al., 1978a), New Zealand rabbits (Schwetz et al., 1973), C57Bl/6 mice (Chapman & Schiller, 1985; Jones & Greig, 1975; McConnell et al., 1978b; Smith et al., 1981; Vos et al., 1974), DBA/2-mice (Chapman & Schiller, 1985), and B6D2F1-mice (Chapman & Schiller, 1985) gave oral LD50 values of 70, 115, 114, 2570, and 296 mg TCDD/kg body weight, respectively. The difference in sensitivity towards TCDD among various strains of mice has been claimed to depend on a genetic variability in the Ah and/or hr-locus (see section 7.8.1).

Male Sherman rats were found to be more sensitive to TCDD than were females (Schwetz et al., 1973), whereas Beatty et al. (1978) reported that male Sprague Dawley rats were more resistant to TCDD than were females. Smith et al. (1981) found adult female C57BL/10 mice to be more resistant to TCDD than adult males of the same strain. No differences in sensitivity to TCDD between sexes were recorded for guinea-pigs (McConnell et al., 1978b; Silkworth et al., 1982) or hamsters (Olson et al., 1980b). Thus data on sex differences in sensitivity to lethal effects of TCDD are conflicting.

Data on the effect of age at exposure to TCDD on the sensitivity of acute response are scarce, and comparisons are hampered by the absence of information, or incomplete information, on the age and/or body weight of the tested animals. However, Beatty et al. (1978) found

that weanling male Sprague Dawley rats were more sensitive to TCDD than were adult males. A dose of 25 µg TCDD/kg body weight caused, after 35 days, a cumulative lethality of 62% in weanling Sprague Dawley rats and 25% in young adults (Christian et al., 1986a). When weanling and mature adults were exposed to a similar toxic dose of TCDD (LD62 and LD60, respectively), onset of death occurred 9 days later in the adults (Christian et al., 1986a).

Schwetz et al. (1973) found LD50 values in rabbits of 115 µg

TCDD/kg body weight after oral exposure, as compared to 275 µg TCDD/kg body weight after dermal exposure. C57Bl/6-mice seem to be more sensitive to ip administration of TCDD (Gasiewicz et al., 1983b) than to oral administration (McConnell et al., 1978b). The LD_{50} value in guinea-pigs was increased from 2.5 µg/kg body weight to 19 µg/kg body weight when the vehicle for the oral administration of TCDD was changed from corn oil to methyl cellulose (Silkworth et al., 1982). Umbreit et al. (1985) compared mortality, and time to death, among guinea-pigs given single oral doses of TCDD in corn oil, TCDD in a suspension of cleaned soil, from an industrial site, or TCDD-contaminated soil from the same industrial site. Animals treated with corn oil, cleaned soil and contaminated site soil survived the 60-day study without any sign of TCDD intoxication. Among animals that received 6 µg TCDD/kg body weight either in corn oil or mixed with cleaned soil, only 3/8 and 1/7 survived, respectively. Deaths occurred between days 9 to 31 and 15 to 25, respectively. These results are different from those reported by McConnell et al. (1984) where TCDD-contaminated soils from Times Beach and Minker Stout were highly toxic to guinea-pigs. LD50 values calculated in this study were 1.75 µg/kg for TCDD in corn oil, 7.15 µg/kg for TCDD in Times Beach soil, and 5.50 µg/kg for TCDD in Minker Stout site soil. The Minker Stout site soil was also potent in inducing AHH-activity in female Sprague Dawley rats. The different results may be due to the vehicle used and/or to the presence of other substances in the soils that may potentiate or retard the TCDD-induced toxicity (Umbreit et al., 1986a). In section 6.4, the results from McConnell et al. (1984) and Umbreit et al. (1985, 1986a) are further discussed from the viewpoint of the "bioavailability" of TCDD in soils (Table 46).

Despite similar routes and vehicles for the administration of TCDD to Golden Syrian hamsters, LD_{50} values varied between 1157 µg/kg body weight (Olson et al., 1980b) and 5051 µg/kg body weight (Henck et al., 1981). A possible explanation for this difference could be the spontaneous occurrence of ileitis observed in the former study, which might have increased the susceptibility of those hamsters to TCDD toxicity.

Table 47. Single lethal dose values for TCDD[a]

Species/strain	Sex/No/	Age/weight	Route/vehicle				
Dose	Duration of	LD_{50}	Time to				

Species/strain (Reference)	Sex/No/ group tested	Age/weight	Duration of observation	Route/vehicle	LD$_{50}$ (µg/kg)	Dose tested (µg/kg)	Time to death (days)
Rats							
Porton (Greig et al., 1973)	F/5-12	8-9 weeks/ 170-200 g	90 days	oral/DMSO	NR	0, 30, 48, 75, 120, 190, 300	40
Porton (Greig et al., 1973)	F/6	9-10 weeks/ 170-200 g	90 days	oral/arachis oil	NR	0, 126, 199, 315, 500	40
Sherman (Schwetz et al., 1973)	M/5-10	NR	2-8 weeks	oral/corn oil acetone (9:1)	22	8, 16, 32, 63	9-27
Sherman (Schwetz et al., 1973)	F/NR	NR	2-8 weeks	oral/corn oil acetone(9:1)	45	NR	13-43

Table 47 (contd).

Species/strain (Reference)	Sex/No/ group tested	Age/weight Duration of observation	Route/vehicle Dose tested (µg/ kg)	LD$_{50}$ (µg/kg)	Time to death (days)

```
Rats (contd)
      Sprague           M/6         adult/NR      ip/olive oil        4
doses      20              60            NR
      Dawley                                                      20-80           60


   (Beatty et al.,
      1978)
      Sprague           F/6         adult/NR      ip/olive oil        4
doses      20              60            NR
      Dawley                                                      10-60


   (Beatty et al.,
      1978)


      Sprague           M/6         25 days/NR    ip/olive oil        4
doses      20              25            NR
      Dawley                                                       5-50


   (Beatty et al.,
      1978)


      Fisher 334N       M/7         11-12 weeks/  oral/corn oil        0
30 days          340ᵇ            28ᵇ
                                   230-280
g                         75                           303ᶜ         26ᶜ
   (Walden
&
150                              164ᵈ           25ᵈ
      Shiller, 1985)                                              225
                                                                  275
                                                                  325
                                                                  375


       CD               M/7         10-11 weeks/  oral/corn oil        0
30 days          297ᵈ            25ᵈ
                                   350-370 g                          75
   (Walden &                                                     150
      Shiller, 1985)                                             225
                                                                 275
                                                                 325
                                                                 375
```

Table 47 (contd).

Species/strain (Reference)	Sex/No/ group	Age/weight	Route/vehicle	Dose tested (µg/kg)	Duration of observation	LD_{50} (µg/kg)	Time to death (days)
Rats (contd)							
Han/Wistar (Pohjanvirta & Tuomisto, 1986)	M/5	NR/300–350	oral/corn oil	1500 2000 2500 3000	39–40 days	> 3000	NR
Mice							
C57BL/6 (Vos et al., 1974)	M/14	3 months/ 23.6–30.8 g	oral/corn oil acetone (6:1)	0 100 150 200	2 months	114	15–30
C57BL/6 (Jones & Greig, 1975)	M/NR	7–15 weeks/ 14–30 g	oral/arachis oil	NR	35 days	126	21±1.6
C57BL/6 (McConnell et al., 1978[b])	M/8	9 weeks/ 21–25 g	oral/corn oil	NR	30 days	284	22–25
C57BL/6J (Gasiewicz et al., 1983b[d])	M/NR	NR	ip/olive oil	NR	30 days	132	NR

Table 47 (contd).

Species/strain (Reference)	Sex/No/ group	Age/weight	Route/vehicle	Dose tested (µg/ kg)	Duration of observation	LD_{50} (µg/kg)	Time to death (days)
Mice (contd)							
C57BL/6J (Chapman & Schiller, 1985)	M/10-15	10-12 weeks/ 22-32 g	oral/corn oil	95 145 190 285	30 days	182	24
C57BL/10 (Smith et al., 1981)	M/5	42-121 days/NR	oral/arachis oil	85 107 135 170 213	45 days	146	22-38
C57BL/10 (Smith et al., 1981)	F/5	42-121 days/NR	oral/arachis oil	85 107 135 170 213 269 338 426 536	45 days	> 450	22-38
DBA/2J (Gasiewicz et al., 1983b[e])	M/NR	NR	ip/olive oil	NR	30 days	620	NR
DBA/2J (Chapman & Schiller, 1985)	M/10-15	10-12 weeks/ 22-32 g	oral/corn oil	1370 1870 2610 3500 4470	30 days	2570	21

Table 47 (contd).

Species/strain (Reference)	Sex/No/ group	Age/weight	Route/vehicle	LD_{50} (µg/kg)	Time to death (days)	Dose tested (µg/kg)	Duration of observation
Mice (contd)							
B6D2F$_1$/J (Gasiewicz et al., 1983[e]	M/NR	NR	ip/olive oil	NR	NR	300	30 days
B6D2F$_1$J (Chapman & Schiller, 1985)	M/10-15	10-12 weeks/ 22-32 g	oral/corn oil	170 220 265 325 425 450	25	296	30 days
Guinea-pigs							
Hartley (Schwetz et al., 1973)	M/NR	NR	oral/corn oil	NR	5-34	0.6	2-8 weeks
Hartley (Schwetz et al., 1973)	M/NR	NR	oral/corn oil acetone (9:1)	NR	9-42	2.1	2-8 weeks
Hartley (McConnell et al., 1978[b])	M/6	3-4 weeks/ 200-250 g	oral/corn oil	NR	17-20	2	30 days

Table 47 (contd).

Species/strain (Reference)	Sex/No/ group	Age/weight	Route/vehicle	Dose tested (μg/kg)	Duration of observation	LD_{50} (μg/kg)	Time to death (days)
Guinea-pigs (contd)							
Hartley (Silkworth et al., 1982)	F/6	NR/500-600 g	oral/corn oil	0.1 0.5 2.5 12.5 20.0	42 days	2.5	32-42
Hartley (Silkworth et al., 1982)	F/6	NR/500-600 g	oral/methyl-cellulose	0.1 0.5 2.5 12.5 20.0	42 days	19	12-42
Rabbits							
New Zealand (Schwetz et al., 1973)	M,F/NR	NR	oral/corn oil acetone (9:1)	NR	2-8 weeks	115	6-39
New Zealand (Schwetz et al., 1973)	M,F/NR	NR	dermal/acetone	31.6 63 126 252 500	3 weeks	275	12-22
New Zealand	M,F/5	NR	ip/corn oil	31.6 63	4 weeks	NR	6-23

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(Schwetz et al.,                                          126
   1973)                                                  252
                                                          500
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Table 47 (contd).

Species/strain (Reference)	Sex/No/ group	Age/weight tested	Route/vehicle	Dose (μg/ kg)	Duration of observation	LD$_{50}$ (μg/kg)	Time to death (days)
Hamsters							
Golden Syrian (Olson et al., 1980[b])	M/5-6	NR/50-80 g	ip/olive oil	0 500 1000 2000 3000	50 days	> 3000	
Golden Syrian (Olson et al., 1980[b])	F/5	NR/50-80 g	ip/olive oil	0 500 1000 2000 3000	50 days	> 3000	14-32
Golden Syrian (Olson et al., 1980[b])	M/5	NR/50-80 g	oral/olive oil	500 1000 2000 3000	50 days	1157	2-47
Golden Syrian (Henck et al., 1981)	M/6	NR/70-120 g	oral/corn oil acetone (9:1)	0 300 600 1000 3000 6000	55 days	5051	9-43

Table 47 (contd).

Species/strain (Reference)	Sex/No/ group	Age/weight	Route/vehicle	Dose tested (µg/kg)	Duration of observation	LD_{50} (µg/kg)	Time to death (days)
Monkeys							
Macaca mulatta (McConnell et al., 1978[a])	F/3	juvenile/ 2.1-2.6 kg	oral/corn oil	0 70 350	47 days	< 70	14-34
Dogs							
Beagle (Schwetz et al., 1973)	M/2	NR	oral/corn oil acetone (9:1)	300 3000	NA	NR	9-15
Beagle (Schwetz et al., 1973)	F/2	NR	oral/corn oil acetone (9:1)	30 100	NA	NR	all animals sur- vived
Chickens							
Leghorn (Grieg et al., 1973)	NR	4-6 weeks/NR	oral/NR	NR	NR	25-50	12-21

[a]   M = male, F = female, NR = not reported, NA = not applicable, ip = intraperitoneal, DMSO = dimethyl sulfoxide.

<pre>
b   supplied by Harlan.
c   supplied by Frederick.
d   supplied by Charles River.
e   based on unpublished studies by Gasiewicz et al., 1983.
</pre>

TCDD affects a variety of organ systems in different species. The organ primarily affected in rodents and rabbits is the liver. In guinea-pigs atrophy of the thymus and lymphatic tissues seems to be the main effect, while dermal effects are prominent signs in non-human primates. Generally it is not possible to specify a single organ whose dysfunction is responsible for death. Overall, TCDD seems to have a predilection for causing pathological changes in epithelial tissues, both cutaneous and internal. This is particularly apparent in non-human primates (Macaca mulatta), and is note-worthy that the lesions mimic to some degree the effects in human beings. The histopathological alterations in tissues include hyperplastic and/or metaplastic alterations as well as hypoplastic responses. The toxic responses of various species to TCDD are summarized in Table 48, adapted from Poland & Knutson (1982). In all animal species studied, death occurred after a time lapse ranging from several days to more than one month after exposure. The delay was dependent on dose but not on species (Table 48). Progressive loss of body weight was a characteristic sign observed in animals given a lethal dose of TCDD. The weight loss became manifest usually within a few days after exposure and resulted in a substantial reduction of the adipose tissue observed at autopsy. At sublethal doses of TCDD a dose-dependent decrease in body weight gain occurred. This TCDD-induced wasting syndrome has been thoroughly investigated in several studies discussed more fully in section 7.4.1.

The greatest difference between species at necropsy, both in gross and histological effects, concerns pathological alterations in the liver. As discussed in detail in section 7.4.2, a dose of TCDD lethal to guinea-pigs did not result in liver damage comparable to the liver lesions described in rabbits and rats or to liver changes observed in mice dying after doses higher than those needed to cause death in these species. In the hamster, frank liver lesions do not occur even after fatal doses.

Chloracne-like lesions can be induced by topical application and/or systemic administration of TCDD in rabbits, non-human primates, and hairless mice. These lesions are further discussed in section 7.4.4.

Severe thymus atrophy was also found at autopsy in all animal species given lethal doses of TCDD. Histological examinations revealed lymphoid cell depletion in thymus cortex, spleen, and lymph nodes. These consistent findings in TCDD poisoning will be discussed in detail in section 7.4.5 together with the other lymphoid tissue-related effects.

Table 48. Specific differences in toxic responses following exposure to 2,3,7,8-TCDD[a, b]

	Rat	Mouse	Rabbit[c]	Chicken[c]	Monkey	Hamster	Guinea-pig	Cow[c]
Hyperplasia and/or metaplasia								
Gastric mucosa	0	0			++	0	0	+
Intestinal mucosa	+							++
Urinary tract					++	++	++	0
Bile duct and/or gall bladder	++			0	++		0	+
Lung: focal alveolar								++
Skin	0	0	++		++	0	0	+[d]
Hypoplasia, atrophy, or necrosis								
Thymus	+	+		+	+	+	+	+
Bone marrow	+			±	+	+		
Testicle					+	+	+	+
Other responses								
Liver lesions	+	+	++	+	+	±	+	+
Porphyria					0		0	+

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++                          +              0
      Oedema                                +              0
0          +                      ++                +
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a   References: monkey (Norback & Allen, 1973; Allen et al., 1977; McConnell
et al., 1978a), guinea-pig (McConnell
      et al., 1978b; Moore et al., 1979; McConnell, 1980; Turner &
Collins, 1983), cow (McConnell, 1980), rat (Kociba et
      al., 1978; Kociba et al., 1979a; McConnell, 1980), mouse (Vos et al.,
1973; Schwetz et al., 1973; McConnell et al.,
      1978b), rabbit (Vos & Beems, 1971; Schwetz et al., 1973), chicken (Allen
& Lalich, 1962; Vos & Koeman, 1970;
      Norback & Allen, 1973; Schwetz et al., 1973), hamster (Olson et al.,
1980b; Henck et al., 1981).

b   Symbols: 0 = lesion not observed, + = lesion observed (number of "+"
denote severity), ± = lesion observed to
      a very limited extent, blank = no evidence reported in literature.
c   Responses followed exposure to 2,3,7,8-TCDD or structurally
related chlorinated aromatic hydrocarbons.
d   Skin lesions in cattle have been observed, but they differ from the
skin lesions observed in other species.

     There are also substantial interspecies differences in the
effects observed in other organs of animals given lethal doses of
TCDD. Icterus was reported in rats (Buu-Hoi et al., 1972a; Gupta et
al., 1973), hepatic porphyria occurred in mice and rats (see section
7.4.3), and ascites with subcutaneous oedema and hydrothorax appeared
in mice (Jones & Greig, 1975; Vos et al., 1974) and monkeys (Allen et
al., 1977). The accumulation of serous fluid in the pericardial sac
occurred in chickens after a single lethal dose of TCDD (see section
7.1.4). Haemorrhages were frequently observed in many organs following
lethal doses in monkeys (Allen et al., 1977), rats, and guinea-pigs
(Gupta et al., 1973). In mice, death was frequently attributed to
terminal haemorrhages (Vos et al., 1974).

     When administered in doses sufficient to cause overt toxicity,
TCDD causes testicular atrophy and degeneration characterized by
reduced spermatogenic activity in mice (McConnell et al., 1978b), rats
(Kociba et al., 1976; Van Miller et al., 1977), and guinea-pigs
(McConnell et al., 1978b). The same symptoms were present in monkeys
fed dioxin-containing toxic fat (Allen & Carstens, 1967; Norback &

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Allen, 1973). The decreases in male sex organ weights (seminal vesicles, ventral prostate, testes, and caput epididymis) were dose-dependent. ED50 values were around 15 mg TCDD/kg body weight in Sprague Dawley rats 7 days after TCDD-treatment when compared to pair-fed control rats (Moore et al., 1985). Shrunken, hyperchromatic nuclei in the two layers of seminiferous tubules closest to the basement membrane were observed in testes of young Sprague Dawley rats 90 h after a single ip dose of 5 mg TCDD/kg body weight (Mittler et al., 1984). Epididymal lesions (Khera & Ruddick, 1973) and decreased amounts of secretory material within the accessory sex glands (Kociba et al., 1976) have been reported in TCDD-treated rats. Reduced prostate weights, both absolute and relative, were found in Han/Wistar rats at non-lethal doses of TCDD (Pohjanvirta & Tuomisto, 1986).

Reduced relative uterine weight, accompanied by decreased mucosa, stroma, and glands, occurred in young C57Bl/6 mice dosed with 6 µg TCDD/kg body weight three times a week for 1 month, but there was no effect on the ovaries (Gallo et al., 1986). TCDD had no effect on the uterine weight in 25 day-old Long Evans rats 2 - 10 days after treatment with single ip doses of 20 or 80 µg/kg body weight (Romkes et al., 1987). However, the increase in uterine weight induced by estradiol treatment was counteracted by simultaneous TCDD-treatment.

Proliferative lesions of the gastrointestinal tract have been found primarily in non-human primates (Allen et al., 1977; McConnell et al., 1978a) whereas proliferative changes of the transitional epithelium in the urinary tract have been found in both guinea-pigs (Gupta et al., 1973; McConnell et al., 1978b) and monkeys (Norback & Allen, 1973).

Reduced or unaffected spleen weight and slight to moderate loss of lymphocytes from spleen germinal centers have been common findings in laboratory animals exposed to sublethal to lethal doses of TCDD (Gasiewicz et al., 1980; Greig et al., 1973; Kociba et al., 1978; McConnell et al., 1978a; Olson et al., 1980b; Vos et al., 1973, 1974). Spleen cellularity in C57BL/6 mice was decreased 14 and 21 days after treatment with 30 µg TCDD/kg body weight (Chastain & Pazdernik, 1985), but not in B6C3F1 or DBA/2 mice 7 days after oral doses of up to 10 µg TCDD/kg body weight (Luster et al., 1984).

The thyroid weight, both absolute and relative to body weight, of the rat has been found to be increased by TCDD-treatment (Bastomsky, 1977; Potter et al., 1983, 1986b). Degenerative changes in the epithelial cells of the thyroid gland were observed in rats after 31 daily oral doses of 1 µg TCDD/kg body weight (Gupta et al., 1973) and 7 days after a single ip dose of 150 µg TCDD/kg body weight (Rozman et

al., 1986). However, Potter et al., (1986b) found no consistent changes in follicle size or colloid content, variation in follicle size, height of follicular epithelium, or resorption of colloid at the periphery of follicles in rats one week after an oral dose of TCDD in the range 6.25 to 100 µg/kg body weight.

Histological changes in the pancreas and in the interscapular brown adipose tissue have been found in Sprague Dawley rats exposed to single ip doses of 150 µg TCDD/kg body weight (Rozman et al., 1986).

Lesions of the adrenal glands have been observed in mice and guinea-pigs treated with (Gupta et al., 1973; McConnell et al., 1978b).

Acute exposure to lethal doses of TCDD has produced minor haematological alterations in all species studied. Anaemia was not observed in mice or guinea-pigs (Zinkl et al., 1973), but a moderate anaemia and leukocytosis occurred in rats (Buu-Hoi et al., 1972a) and monkeys (McConnell et al., 1978a). On the contrary, haemoconcentration was observed in rats after exposure to a somewhat lower but still lethal dose of TCDD (Greig et al., 1973; Zinkl et al., 1973). Thrombocytopenia and clotting abnormalities were observed in rats after acute exposure to lethal levels of TCDD (Weissberg & Zinkl, 1973). Hypocellularity of the bone marrow was found in guinea-pigs (McConnell et al., 1978b), rhesus monkeys (Allen et al., 1977; McConnell et al., 1978a), and mice (McConnell et al., 1978b). However, in these studies decreased bone marrow cellularity, as judged by histological examination, appeared only at doses high enough to cause severe toxicity in the experimental animals. More recent studies (Chastain & Pazdernik, 1985; Luster et al., 1980, 1985) have demonstrated that collection and enumeration of bone marrow cells in suspension provides a more sensitive and quantitative method than histological preparation for assessing bone marrow cellularity.

Decreased bone marrow cellularity was found in adult male C57Bl/6 mice 3 days after exposure to 120 µg TCDD/kg body weight (Chastain & Pazdernik, 1985), and in female B6C3F1 mice 5 days after exposure to 10 µg TCDD/kg body weight (Luster et al., 1985), but not in female DBA/2 mice, even at a dose of 50 µg TCDD/kg body weight (Luster et al., 1985). The myelotoxic potential of TCDD is described in section 7.4.6.

Changes in clinical chemistry, including serum enzyme activities, serum protein concentrations, and lipid levels, observed in animals after acute exposure to TCDD, primarily reflect damage to other organ systems, mainly the liver (McConnell et al., 1978a; McConnell & Moore,

1979; Olson et al., 1980b). Liver-related enzyme activities in serum are affected in those animal species where liver damage is a prominent sign of TCDD toxicity. In those animal species where hepatotoxicity is not as apparent, such as monkeys and guinea-pigs, these enzyme activities are essentially normal. Also the TCDD-related decrease in serum albumin seems to be secondary to the hepatotoxic effect, since the decrease is less evident or non-existent in those animals that show little liver damage. Generally serum triglycerides and free fatty acid levels are increased after TCDD exposure, while that of serum cholesterol is decreased. However, marked species differences exist and again these effects seem to be secondary to liver damage. For further details on hyperlipidaemia, see section 7.4.7.

Indicators of renal damage such as blood urea nitrogen, creatinine, and blood electrolytes are usually within normal limits after TCDD exposure.

In view of the interspecies differences in the organ distribution of TCDD and the variability in the effects on various organs in the different species, the effects of TCDD in non-human primates are of particular interest. Nine female juvenile rhesus monkeys (Macaca mulatta) were divided into three groups of three and given TCDD in a single oral dose of 0, 70, or 350 µg per kg/body weight (McConnell et al., 1978a). The first indication of a toxic effect of TCDD, at day 3, was weight loss, followed by periorbital oedema, conjunctivitis, and thickening of the Meibomian glands on day 12. Subsequently, the eye lashes, facial hair, and toe and finger nails were lost. Monkeys given the highest dose showed a moderate absolute lymphopenia and thrombocytopenia. Serum cholesterol levels dropped, while the serum triglyceride levels increased. Alkaline phosphatase and total bilirubin were normal, but glutamic oxalic transaminase and aldolase were increased. A decrease in the albumin fraction of the total serum protein was noticed. The three monkeys given TCDD in a dose of 350 µg/kg body weight died or became moribund between days 28 and 34 after administration. One of the monkeys given TCDD in a dose of 70 µg/kg body weight died 14 days after administration. At autopsy body fat was almost completely absent in all treated monkeys. Ascites was noticed

in two animals and all monkeys had markedly distended and thickened bile ducts and gall-bladders. Small focal ulcerated areas in the fundus of the stomach were observed in two monkeys. Microscopic examination showed that the Meibomian glands were dilated and filled with keratinaceous debris. Squamous metaplasia of the glandular portion, with atrophy of sebaceous cells, was present. Occasional scattered necrotic hepatocytes were noted in the liver on microscopic examination. The gastric ulcers that were found extended into the

lamina propria.

Schwetz et al. (1973) reported that no signs of toxicity were
observed in four male mice and two female rats given single oral doses
of respectively 2 and 1 g 2,7-diCDD/kg body weight (purity
99.6-99.8%). For octaCDD (purity 98.86%), oral doses of 1 g/kg body
weight to five female rats and 4 g/kg body weight to four male mice
did not cause any toxic symptoms. A sample containing two different
isomers of hexaCDD (purity > 99%, 89:11) killed 1 of 2 and 0 of 2
male rats when given as single oral doses of 100 and 10 mg,
respectively. The only toxic sign observed among these rats was loss
of body weight. These studies lasted for 2 to 8 weeks.

The toxicities of single oral doses of nine PCDDs, including
TCDD, in C57BL/6J mice and Hartley guinea-pigs were compared by
McConnell et al. (1978b) (Table 49). The purity of the various isomers
tested exceeded 97%. Groups of eight male mice and of six male
guinea-pigs were used for each dose of any of the tested compounds,
and the animals were followed for 30 days after administration. The
toxic effects observed after administration of the different PCDDs
were similar, the only difference among the congeners being the amount
needed to produce a given effect. Progressive decrease of body weight,
more pronounced in guinea-pigs than in mice, was observed after a
lethal dose of any of the congeners tested. Marked reduction in
deposited adipose tissue deposits was a constant finding in animals
given a lethal dose of any of the PCDDs. Reduction of muscle mass and
severe dehydration were observed in guinea-pigs, and ascites,
subcutaneous oedema, and hydrothorax in some of the treated mice.
Decrease of thymus weight was a constant finding in both species,
being more pronounced in mice and in the guinea-pigs that died.
Histological examination of the thymus of the guinea-pigs that died as
early as 5 days after administration of PCDDs revealed scattered
necrosis of lymphocytes throughout the cortex, with concomitant
phagocytosis by macrophages. This was even more apparent in animals
that died 14 days after administration, in which a noticeable decrease
in the thickness of the cortex was observed. In the animals that had
died by day 20 it was difficult to differentiate the cortex from the
medulla, but little evidence of necrosis was present.

Table 49. Estimated single oral LD$_{50}$ values for some PCDDs[a]

| Chlorination of PCDDs | Guinea-pigs (mg/kg)[b] | Mice (mg/kg)[b] |
| --- | --- | --- |

```
2,8   > 300 000   NR
2,3,7   29 444   > 3000
2,3,7,8   2   284
1,2,3,7,8   3   338
1,2,4,7,8   1125   > 5000
1,2,3,4,7,8   73   825
1,2,3,6,7,8   70-100c   1250
1,2,3,7,8,9   60-100c   > 440
1,2,3,4,6,7,8   > 600   NR
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a       From: McConnell et al. (1978b).

b       Spearman-Karber method.

c       Estimated range due to variability in replicates.

NR    = not reported.


        In guinea-pigs that survived 30 days after a lethal dose of any
of the congeners, the thymus was reduced to one-fourth of its size in
controls. However, thymus histology at this time was often
comparatively normal. A reduction of the lymphoid follicles in the
spleen and of the Peyer's patches in the intestine was observed with
less conspicuous necrosis, which again was not evident in the 30-day
survivors. Striking hypocellularity was found in the sternal bone
marrow in the guinea-pigs that died, but was less obvious in
survivors. Similar thymic and splenic changes were found in mice.
However, bone marrow atrophy occurred only rarely in this species and
then it was less pronounced than in guinea-pigs. In the guinea-pigs
that died, and occasionally in survivors, a marked hyperplasia of the
renal pelvis was observed, invariably extending into the ureter and at
times involving the urinary bladder mucosa. Gastrointestinal
haemorrhages and occasional microscopic dilatation of crypts in the
glandular portion of the gastric mucosa were observed in dead
PCDD-treated animals of both species. Retro-orbital haemorrhages with
exophthalmus and haemorrhages with detachment of the retina were seen
in mice that died after a lethal dose of any of the congeners tested.
Adrenal haemorrhages and moderate atrophy of the zona glomerulosa were
seen in guinea-pigs that died. These changes were not observed in mice
or in surviving guinea-pigs. In guinea-pigs, primarily in animals that
died during the observation period, changes in the spermatogenic
epithelium were observed, with testicular tubules containing only
spermatogonia and Sertoli cells in severely affected animals. Reduced
spermatogenesis, necrotic spermatocytes, and spermatozoa within the

lumen of the testicular tubules and in the epididymis, and

multi-nucleated giant cells within the seminiferous tubules, were found in the mice that died but not in those that survived. On the other hand, liver lesions were observed with the same frequency and degree of involvement in all the mice given the same dose, whether they died or survived. Minimal liver changes were detected in guinea-pigs, changes largely confined to central congestion with occasional degeneration of hepatocytes in dead animals. Fluorescence, as an indication of porphyria, was found only in mice, particularly in the liver but also in the incisors, cranial bones, costochondral junction, and stifle joint. It was dose related and detectable at doses several times less than the $LD_{50}$.

Haemolysis and hyperproteinaemia were found in dying animals of both species. In mice surviving 30 days, but not in guinea-pigs, the blood a-globulin level was decreased, with a resultant increase in the albumin/globulin ratio.

With all PCDDs for which it was possible to establish an LD50 values were in the range 10 to 100 times lower in guinea-pigs than in mice. To produce the greatest toxicity the lateral positions 2,3,7, and 8 must be fully chlorinated. With 2,3,7-triCDD or 2,8-diCDD the $LD_{50}$ values were in the range 1000 to 100 000 times higher than with TCDD. The addition of a chlorine atom at an _ortho_-position, e.g., 1,2,3,7,8-pentaCDD, resulted in only a minor reduction of toxicity. An additional chlorine atom further reduced the toxic potency but the $LD_{50}$ values of 1,2,3,4,7,8-hexaCDD and 1,2,3,6,7,8-hexaCDD remained comparatively low. The toxicity of 1,2,3,4,6,7,8-heptaCDD was further reduced. A reduction in the rate of weight gain was observable in guinea-pigs given doses of this compound exceeding 200 µg/kg body weight. However, no deaths were observed during the experiment even at doses as high as 600 µg/kg body weight.

The acute oral toxicities of soot and benzene extracts of soot, containing PCDDs and PCDFs from a fire in a PCB-containing transformer (Binghamton, New York, USA) were determined to be 410 mg/kg body weight and 327 mg equivalents/kg body weight, respectively, in female Hartley guinea-pigs (Silkworth et al., 1982). The observation period was 42 days. The test substances were given in 0.75% aqueous methyl cellulose and the doses used were 250, 500, 750, 1000, and 1250 mg soot/kg body weight and benzene extracts corresponding to 4, 20, 100, 500, and 1000 mg soot/kg body weight. An extensive investigation, including pathology, haematology, and serum chemistry alterations, in groups of six male and female Hartley guinea-pigs, 42 days after single oral doses of 1, 10, 100, or 500 mg Binghamton soot/kg body weight in 0.75% methyl cellulose, was reported by Silkworth et al.

(1982). Control animals received 500 mg activated carbon/kg body
weight in the same vehicle. No treatment-related differences were
observed at 1 and 10 mg soot/kg body weight. Decreased body weight
gain occurred in both sexes at 100 and 500 mg/kg, and decreased thymus
weight occurred at 500 mg/kg for males and at 100 and 500 mg/kg for

females. The kidney weight was decreased only in males at 100 and 500
mg/kg. There were no treatment-related alterations in haematological
values. Male guinea-pigs had significantly increased serum
triglyceride levels at 100 and 500 mg/kg and females at 500 mg/kg
only. Elevated aspartate amino transferase (at 100 and 500 mg/kg) and
decreased Y-glutamyltransferase (at 500 mg/kg) were observed in the
serum of female guinea-pigs. The only clearly dose-related
microscopical findings in soot-exposed guinea-pigs were metaplasia of
salivary gland interlobular duct epithelium and goblet cell
hyperplasia of pancreatic interlobular ducts. These lesions occurred
only in males at doses of 100 or 500 mg soot/kg body weight.
Microscopic lesions, which tended to be more frequent and/or severe in
treatment groups than in controls, included bile duct hyperplasia,
hepatocellular cytoplasmic inclusions, vacuolation of the adrenal
cortex, and focal lacrimal adenitis.

7.1.2  In vitro studies on mammalian cells

     Over 30 cell types, including primary cultures and cells from
established and transformed cell lines derived from various tissues of
at least six animal species, have been examined for their response to
TCDD (Beatty et al., 1975; Knutson & Poland, 1980a; Niwa et al., 1975;
Yang et al., 1983). The effects studied were viability, growth rate,
and morphological alterations. No toxic effects were observed except
in one rat hepatoma cell line in which Niwa et al. (1975) reported
decreased viability after exposure for 72 h to TCDD at a concentration
of 300 nmol/litre. This concentration is high when compared to the
$LD_{50}$ in rats and mice. No effect on these cells was observable at 30
nmol/litre.

     The biochemical responses found in primary hepatocytes from rats
exposed to 10 µg TCDD/kg body weight in vivo was not present when
the hepatocytes were exposed in vitro to TCDD at 50, 100, or 200
nmol/litre for 48 h (Yang et al., 1983a).

7.1.3  Studies on birds

     Chick oedema disease first gained attention in the United States
in 1957. An extensive outbreak among chickens occurred in that year in
Georgia (Firestone, 1973; Sanger et al., 1958; Simpson et al., 1959).

The cause of the disease was traced to the presence in the feed of toxic components later identified as chlorinated dibenzo-p-dioxins. TCDD was identified as one of the isomers in the mixture of chlorinated dibenzo-p-dioxins capable of producing chick oedema (Flick et al., 1973).

Clinical signs of chick oedema disease consist of dyspnea, reduced body weight gain, stunted growth, subcutaneous oedema, pallor, and sudden death. In young chickens gasping was the first noticeable sign, followed by a waddling gait. Gross inspection of the birds at autopsy revealed an increased amount of fluid in the pericardial sac

and pale livers with a mottled and irregular granular surface. In advanced stages the chickens developed a distended abdomen filled with fluid. Endotheliosis of the vascular system was observed at microscopic examination, as well as pronounced proliferation of the endothelium of the glomerular capillaries and necrosis of the liver cells. Diseased chickens developed pulmonary oedema and perivascular lymphocyte infiltration, as well as oedema of the cardiac muscle with interstitial lymphocytic infiltration (Allen, 1964; Allen & Lalich, 1962, McCune et al., 1962; Simpson et al., 1959).

Experimentally, chick oedema has been produced with a single dose of 25 to 50 µg TCDD/kg body weight (Greig et al., 1973). When mixtures of tri- and tetraCDDs were fed at dietary concentrations of 0.01 µg/kg, the chickens developed oedema and 83% of them died (Flick et al., 1972).

Potency for inducing chick oedema was compared for three different PCDDs: TCDD (purity 91% and > 99%), hexaCDD (purity > 99%, two isomers), and octaCDD (purity 98.86%) (Schwetz et al., 1973). In the experiments, 3-day-old white Leghorn cockerels were exposed for 20 to 21 days to one of these congeners at several dose levels. Chick oedema occurred in birds given oral doses of 1 or 10 µg TCDD/kg per day, or of 10 or 100 µg hexaCDD/kg per day. Chick oedema was not observed in chicks maintained on a diet containing 0.1 or 0.5% octaCDD. The weight of the Bursa of Fabricius was significantly decreased in 2-week-old white Leghorn cockerels decapitated 2 days after three daily ip doses of 10 µg TCDD/kg body weight (Sawyer et al., 1986).

7.1.4  Toxicity of metabolites

Not only has identification of several in vivo metabolites been achieved, but the acute oral toxicity of TCDD metabolites excreted in the bile of dogs has been studied in the guinea-pig (Weber et al.,

1982a). 3H-labelled TCDD was administered directly into the duodenal lumen of two 1-year-old Beagle dogs in four portions of 1 to 2 mg at time intervals of 2 to 7 days. Excretion of radioactive material from pooled bile samples collected daily for 4 or 7 days, and thereafter in pooled samples of 2 or 3 days, was performed with a method yielding about 50% of the total radioactivity of the bile. The extracts containing the metabolites were concentrated and dissolved in 1,3-propanediol for administration. Male Pirbright guinea-pigs were used in a 5-week toxicity study. Five animals in each of four dose-groups were given a single oral dose via gastric intubation. The amount of TCDD metabolites was calculated by means of radioactivity measurements to be 0.6, 6.0, 30.0, or 60.0 µg/kg body weight. Three animals, one control and two in the high-dose group, died within 48 h after administration. One death in the high-dose group was due to gastric perforation during dosing. The other animals which died exhibited histological lesions which, according to the authors, were due to material coextracted from bile together with the metabolites.

The light microscopic examination of the liver, spleen, pancreas, thymus, kidneys, lungs, and adrenals revealed no histological changes, and no other toxic effects due to the metabolites of TCDD could be recorded in this study. The authors concluded that TCDD metabolites from the dog are at least 100 times less toxic to male guinea-pigs than TCDD itself.

When the metabolites 2-hydroxy-3,7,8-triCDD and 2-hydroxy-1,3,7,8-tetra CDD were given as single ip injections of 100, 1000, or 5000 µg/kg body weight to young male Wistar rats, no decrease in body weight gain was noted and thymic atrophy was not seen 14 days later (Mason & Safe, 1986). When compared to TCDD, 1-hydroxy-3,7,8-triCDD was at least 3 orders of magnitude less effective in inducing hepatic AHH and EROD activities, whereas 2-hydroxy-1,3,7,8-tetra CDD was inactive at all dose levels tested.

7.1.5  Modulation of the acute toxicity

Several studies have been performed which attempt to modify the acute toxicity of TCDD. Manara et al. (1984) studied the effect of activated charcoal or cholic acids in the diet on the mortality after 60 days and mean time to death in mice, rats, and guinea-pigs exposed to TCDD. Dietary levels  of charcoal (2.5%), cholic acid (0.25%), and dehydrocholic acid (0.5%) decreased the mortality induced in C57Bl/6 mice by  a single sc dose of 110 mg TCDD/kg body weight from 93% to 53,  21, and 53%, respectively. The mean time to death was prolonged from 29 days with a normal chow diet to 35-48 days with the above dietary additives. In CD rats the addition of  charcoal (2.5%) and

cholic acid (0.15%) decreased mortality from 80% to 50 and 70% respectively. The mean time to death was not affected. Charcoal (5%) added to guinea-pig chow decreased TCDD induced mortality from 64% to 29% and reduced the mean time to death from 29 to 14 days. These additions to  the diet did not prevent body weight loss or liver enlargement in the mice or guinea-pigs (Manara et al., 1984), but 5% charcoal in the diet protected against TCDD-induced thymus athrophy in C57Bl/6 mice 14 days after a single oral dose of 10 mg TCDD/kg body weight (Manara et al., 1982). The addition of 5% n-hexadecane in the diet increased the TCDD induced mortality from 60% to 100% and the mean time to death, within the 50 day study, from 19.8 to 28.8 days in Sprague Dawley rats treated with a single ip dose of 60 mg TCDD/kg body weight (Rozman, 1984). N-hexadecane itself did not affect animal viability.

Increased survival times have been demonstrated in mice receiving daily injections of triiodothyronine (T3) after TCDD treatment (Neal et al., 1979) and in rats thyroidectomized before TCDD treatment (Rozman et al., 1984). Although thyroidectomy increases the mean time to death, it does not prevent TCDD-related mortality in rats (Rozman et al., 1985a). Thyroidectomy has been demonstrated to counteract TCDD-induced decreases in thymus weight and to reduce the spleen

plaque-forming cell response (Pazdernik & Rozman, 1985). A number of hepatic enzyme activities were induced by TCDD to an equal magnitude in thyroidectomized and normal rats (Rozman et al.,  1985b). It has been suggested that TCDD-treatment of rats leads to hypothyroidism, a possible protective mechanism against TCDD toxicity (Pazdernik & Rozman, 1985). However, available data on changes in T3, thyroxine (T4), and thyroid-stimulating hormone (TSH) levels in TCDD-treated rats are not sufficient to state whether the animals are functionally hypothyroid, euthyroid, or hyperthyroid. Results presented by Potter et al. (1986b) (see section 8.4.9) suggest  that TCDD-treated rats remain essentially euthyroid and that the altered thyroid status is neither a major contributor to TCDD toxicity nor a key response to TCDD exposure.

Daily injections of butylated hydroxyanisole protected against TCDD-induced lethality in female Sprague Dawley rats, whereas vitamins E and A, two other antioxidants, did not (Hassan et al., 1985a,b). Dietary selenium, if given in an optimal dose, provides partial protection from the lethal effects of TCDD in female Sprague Dawley rats (Hassan et al., 1985c). None of these treatments could counteract the TCDD-induced body weight loss (Hassan et al., 1985a,b,c).

## 7.2  Short-term Toxicity

7.2.1  Studies on rats

    Data from the earliest subchronic laboratory study, in which rats
were exposed to daily and/or weekly doses of TCDD,  were reported in
four separate papers (published simultaneously) covering general
effects (Harris et al., 1973), pathology (Gupta et al., 1973),
haematological and clinical chemistry changes (Zinkl et al., 1973),
and immuno-biological effects (Vos et al., 1973). Female CD rats were
given TCDD by  gavage in daily doses of 0.1, 1, or 10 µg/kg body
weight for 31 days. The body weight of animals exposed to the highest
dose started to decrease within the first week of exposure and  15/16
animals died or became moribund 17 to 31 days after administration of
the compound began. Pathological changes were comparable to those
observed in rats given a single lethal dose, and included severe
thymic atrophy and liver damage, icterus, haemorrhages in various
organs, and the depletion of lymphoid organs. Weight gain was also
reduced at the daily dose level of 1 µg/kg body weight. However, there
were no deaths and in the animals that were killed moderate thymic
atrophy, slight to moderate liver damage, and, in some  of the
animals, degenerative changes in the kidneys and in the  thyroid gland
were reported. The weight gain was not affected  and significant
histopathological changes were not found in rats that received 0.1
µg/kg body weight per day. A decrease in thymic weight that was
significant on day 24, was observed at the lowest exposure level
(Gupta et al., 1973; Harris et al., 1973). Blood samples were
collected 3, 6, 10,  13, 17, 24, and 31 days after administration of

TCDD began. Serum enzyme activities related to liver damage began to
increase after 10 days of exposure and remained high until death
occurred in the 10 mg TCDD/kg per day group. This group  of animals
also exhibited increased serum bilirubin levels commencing on day 13.
These parameters were only slightly affected in rats receiving 1 µg
TCDD/kg per day. Thrombocytopenia occurred at all dose levels. After
3 days of  treatment with either 1 or 10 µg/kg per day, animals had
depressed platelets counts that remained low throughout the study. In
the low-dose rats, platelets were significantly decreased by day 17.
No significant leukocytopenia or lymphocytopenia occurred in rats at
any dose level. These results are in good agreement with the results
from a more detailed haematological study on female CD rats given
daily oral doses of 10 µg TCDD/kg body weight for 10 and 14 days
(Weissberg & Zinkl, 1973).

    When oral doses of 0.02, 1.0, or 5.0 mg TCDD/kg body weight were
given weekly to groups of 10 female CD rats for 6 weeks, all the
animals survived (Harris et al., 1973). However, body weight gain

decreased in the 5.0 µg/kg group during  the exposure period, and at
the end of this period the thymus/body weight ratio was approximately
50% of the ratio found in the controls. Liver damage was reported as
slight at this dose level. No effect on body or thymic weight and no
significant histopathological changes were observed in rats given
1 µg/kg body weight or less.

    Adult male and female Sprague Dawley rats, in groups of 12, were
given 0, 0.001, 0.01, 0.1, and 1.0 µg TCDD/kg body weight by gavage,
5 days per week for 13 weeks (Kociba et al., 1976). At the end of the
treatment period, five rats of each sex were killed for
histopathological examination, while the remaining animals were
retained for post-exposure observation. Doses of 1 µg TCDD/kg body
weight per day caused five deaths in females, with three occurring
during treatment and two after treatment, and two deaths in males,
both occurring in the post treatment period. Decreased body weights
and food consumption were found at the two highest dose levels both in
males and females. Decreased relative thymus weight and increased
relative liver weight occurred only in the males given the highest
dose but in both the 0.1 and 1.0 µg/kg female groups. Male rats had
significantly depressed haematological values (packed cell volume, red
blood cell count, and  haemoglobin) in the two high-dose groups, while
these values were normal in all female rats. Gross, as well as
histological, examination revealed treatment-related effects only in
the high-dose groups with some minor findings in the 0.1 µg/kg  group.
Subcutaneous oedema, decreased sizes of testes and uteri, and a
decreased number of corpora lutea were found at necropsy. Histological
findings were limited to lymphoid tissues, liver, and epithelial
linings. The lymphoid tissues, including thymus, were depleted of
lymphocytes. The liver of both male and female rats showed pleomorphic
and multinucleated hepatocytes. Foci of necrosis, with focal
reticuloendothelial aggregations in the areas of parenchymal cell
necrosis, were observed. Hyperplasia of Kupffer cells and an increased
amount of a golden-brown pigment were noted. The hepatic changes were
more pronounced near the periphery of the lobules. Slight hyperplasia

of bile ducts and ductular epithelium was present. The uterus was
lined by cuboidal epithelium in the female rats. The rats given 0.01
mg TCDD/kg did not differ from the controls in any of these
parameters, except for a slight increase in the mean liver to body
weight ratio.

    Goldstein et al. (1982) exposed groups of eight female Sprague
Dawley rats to 16 weekly oral doses of 0, 0.01, 0.1, 1.0, and 10.0 µg
TCDD/kg body weight in a study (further discussed in section 8.4.3)
primarily aimed at investigating TCDD-induced porphyria. All animals

given the highest dose died, or were moribund after eight to twelve doses and were killed. A decrease in body weight gain was seen in this group within one week of treatment. Decreased body weight gain was observed also in the 1.0 µg TCDD/kg per week group, but not until several weeks after the start of treatment. Hepatic porphyria was found in 7 out of 8 animals receiving weekly doses of 1.0 µg TCDD/kg body weight, in 1 out of 8 receiving 0.1 µg/kg per week, and in none of the animals receiving 0.01 µg/kg per week or the lethal dose of 10.0 µg/kg per week. Porphyria was not reversed after six months recovery from a 16-week exposure to 1.0 µg/kg body weight per week.

Feeding male Wistar rats (110 g) 0, 0.2, or 1.0 µg octaCDD/kg diet for two weeks, resulting in a total intake of 0, 22.7 (± 1.0) or 120.7 (± 2.8) mg octaCDD, had no effect on body weight gain, feed consumption, or tissue weights (liver, thymus, testes, heart, and kidney) (Williams et al., 1972). Congestion of the liver occurred in the high-dose group.

Daily doses of 100 mg octaCDD for 21 days produced no effects on appearance, activity, or eating habits in male Sprague Dawley rats, but slightly increased relative liver weight. A moderate increase in the hepatic smooth endoplasmic  reticulum was noted (Norback et al., 1975).

7.2.2  Studies on mice

Oral doses of 0.2, 1, 5, or 25 µg TCDD/kg body weight were given in corn oil to male C57Bl/6 mice weekly for four weeks (Harris et al., 1973; Vos et al., 1973). One animal of the 25 µg/kg group died after 24 days. Significant weight loss was observed only in the high-dose group. Thymic atrophy, characterized by nearly complete loss of cortex, occurred in the 5 and 25 µg TCDD/kg body weight groups.

7.2.3  Studies on guinea-pigs

All 10 female Hartley guinea-pigs that received weekly oral doses of 1 µg TCDD/kg body weight died, or were killed when moribund, between days 24 and 32 after the first dose (Gupta et al., 1973; Harris et al., 1973; Vos et al., 1973). Light microscopic findings of moribund or dead animals revealed severe atrophy of the cortex of the thymus with destruction of lymphocytes. There was lymphoid cell

depletion  in spleen and lymph nodes. Haemorrhages, mitotic figures, and  loss of lipid vacuoles were observed in the adrenals. Liver effects were restricted to diffuse single-cell necrosis, predominantly in the periportal area. Haemorrhages were found in the urinary bladder

and gastrointestinal tract. The lymphocyte count was decreased at all doses, whereas total leukocyte values were decreased at doses > 0.04 µg/kg. Animals that received eight weekly doses of 0.008, 0.04, or 0.2 µg TCDD/kg body weight all survived. At the 0.2 µg/kg dose level, decreased body weight gain and decreased relative thymus weight were observed.

A 90-day feeding study of TCDD in male (250-370 g) and female (230-340 g) Hartley guinea-pigs was performed by DeCaprio et al. (1986) and included extensive pathology, haematology, and serum chemistry on surviving animals. The diets contained 0, 2, 10, 76, or 430 ng TCDD/kg. Animals that received the highest dose exhibited severe body weight loss, decreased feed consumption, and mortality. When 60% mortality was reached, on day 46 for males and day 60 for females, the remaining animals in these groups were sacrificed. The estimated total TCDD consumption at that time was 1.3 and 1.9 µg TCDD/kg body weight for males and females, respectively. No treatment-related mortality was observed at the 76 ng/kg dose level, which corresponded to a total estimated intake of 0.44 µg TCDD/kg body weight over the 90 days. Decreased body weight gain and increased relative liver weight were seen in both sexes, whereas reduced relative thymus weight occurred in males only. At doses of 2 and 10 ng/kg diet, no dose-related alterations were observed. The only treatment-related effect on haematology and serum chemistry parameters was the elevation of serum triglycerides for male guinea-pigs at the 76 ng/kg dose level. The presence of hepatocellular cytoplasmic inclusion bodies in female guinea-pigs was the only significant microscopical finding, except for thymus atrophy. Based on this study, a no-observed-effect level of 0.6 ng TCDD/kg body weight per day in guinea-pigs was estimated.

DeCaprio et al. (1986) also followed the body weight changes and mortality during and after feeding male Hartley guinea-pigs (250-360 g) a diet containing 430 ng TCDD/kg. The diet was fed for 11, 21, or 35 days and was then withdrawn during a 79, 69, or 55-day recovery period. The rate of change of weight per day was the same as that of animals on a control diet, after an initial weight loss of approximately 10% during the first 5 days. When animals were fed the TCDD-diet for 21 and 35 days, a significant mortality, 10% and 70%, respectively, was apparent. Both body weight gain and absolute body weight were severely depressed in surviving animals throughout the study. Animals destined to die generally lost more than 20% of the original weight, whereas less pronounced weight losses were usually followed by increases in body weight during the recovery period.

A PCB-containing transformer fire at the State office building,

Binghamton, New York, USA, resulted in contamination of the building with soot-like material containing various PCDDs and PCDFs (see section 4.5.10). This soot, mixed with diet, was fed to groups of 10 male (250-350 g) and female (200-350 g) Hartley guinea-pigs for 90 days (0, 0.2, 1.9, 9.3, and 46.3 mg soot/kg diet) or 32 days (231.5 mg soot/kg diet) (DeCaprio et al., 1983). The total intake of soot during the study corresponded to approximately 0.3, 3, 13, 67, and 100% of the $LD_{50}$ dose of Binghamton soot in guinea-pigs (Silkworth et al., 1982) (see section 8.1.1.1). A dose-related decrease in body weight gain occurred at 9.3 and 46.3 mg soot/kg diet. Body weight loss and decreased feed intake was evident in the animals given 231.5 mg soot/kg diet. Three male and three female guinea-pigs given 46.3 mg soot/kg died. Seven animals in the highest-dose group died within 28 to 31 days and the remaining moribund animals were killed on day 32. Gross pathology revealed no effects at 0.2 mg soot/kg diet. Thymic atrophy occurred only in males at 1.9 mg soot/kg diet, but in both sexes at higher doses. The relative spleen weight was significantly increased at 46.3 mg TCDD/kg in both sexes. Treatment-related microscopical findings included metaplasia of salivary gland epithelia (> 1.9 mg soot/kg diet), increased number of goblet cells in pancreatic ducts (> 46.3 mg soot/kg diet for males), focal lacrimal gland adenitis (> 9.3 mg soot/kg diet for males and > 46.3 mg soot/kg diet for females), depletion of haematopoietic cells from the bone marrow (> 46.3 mg soot/kg diet for females, > 231.5 mg soot/kg diet for males), and hepatocellular cytoplasmic inclusion bodies (9.3 and 46.3 mg soot/kg diet in both sexes). Fatty infiltration of the liver, reduced thickness of thymic cortex, and degenerative changes of the stomach and intestine were observed only in high-dose animals. Haematological alterations were observed only in animals at the 46.3 mg soot/kg diet dose level, whereas alterations in serum chemistry values were found also at lower levels. Toxic effects of feeding Binghamton soot for 90 days were similar to the effects occurring after acute exposure (Silkworth et al., 1982) (see section 8.1.1.1), but the effects were seen at a lower total dose after subchronic exposure than after acute dosing. The effect seen at the lowest level in this study was thymic atrophy at 1.9 mg soot/kg diet, which was equivalent to 7.8 ng TCDD/kg body weight per day. A comparison between the effects of feeding pure TCDD and TCDD-contaminated soot to guinea-pigs (DeCaprio et al., 1986) demonstrated that pure TCDD produced less variability of alterations and gave a steeper dose-response relationship for many effects. Exposure to Binghamton soot characteristically resulted in salivary gland duct metaplasia and decreased serum sodium and potassium levels (DeCaprio et al., 1983).

When male guinea-pigs (200 g) were fed a diet containing 2.5% HCl-pretreated fly ash from a municipal incinerator (Zaanstad, The

Netherlands) for up to 95 days, the animals exhibited progressive
weight loss, hair loss, and increased relative liver weight (van den
Berg et al., 1986b). One animal died on day 76.

Table 50. Studies on long-term exposure (excluding cancer studies) to TCDD
in laboratory animals

| Species/Strain Treatment schedule | Sex/number/ group[d] | Doses | Parameters monitored |
|---|---|---|---|
| **Rats** | | | |
| Sprague Dawley[a] in diet continuously for 65 weeks | M/10 | 0 ng/kg<br>1 ng/kg<br>5 ng/kg<br>50 ng/kg<br>500 ng/kg<br>1000 ng/kg<br>5000 ng/kg<br>50 000 ng/kg<br>500 000 ng/kg<br>1 000 000 ng/kg | survival |
| Sprague Dawley[b] in diet continuously for 2 years | M and F/10 | 0.001 µg/kg per day<br>0.01 µg/kg per day<br>0.1 µg/kg per day | extensive histopathology, haematology and clinical chemistry |
| **Mice** | | | |
| Swiss by gavage weekly for 1 year | M/38-44 | 0 µg/kg/week<br>0.007 µg/kg per week<br>0.7 µg/kg per week<br>7.0 µg/kg per week | histopathology |

Monkeys

Macaca mulatta[c]    F/8    500 ng/kg continuous in      extensive

the

diet for      histopathology,

9

months      haematology and

clinical chemistry

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[a] Van Miller et al. (1977). [b] Kociba et al. (1978, 1979a,b).
[c] Allen et al. (1977).    [d] M = male; F = female.

### 7.2.4 Studies on hamsters

No toxic effects were reported in male Golden Syrian hamsters (50-70 g) given a diet containing 2.5% HCl-pretreated fly ash from a municipal incinerator in Zaanstad, The Netherlands for up to 95 days (van den Berg et al., 1986b).

### 7.2.5  Studies on monkeys

A cumulative dose of 0.2 µg TCDD/kg body weight, divided into nine oral doses given three times per week, produced no clinical toxicity in female rhesus monkeys (Macaca mulatta) (McNulty, 1984). However, clearly toxic signs did occur in those monkeys that received cumulative doses of 1.0 and 5.0 µg TCDD/kg body weight over the same time period. The first signs were thickening and reddening of the eyelids, followed by weight loss, dryness and granularity of the skin, and loss of hair, and in some cases anaemia, purpura, and bleeding from the nose and mouth. Animals that died showed squamous metaplasia of the sebaceous glands, mucous metaplasia of the gastric mucosa, hyperplasia of biliary ductal epithelium, gingivitis, and hypoplasia of the bone marrow. The times to death after dose were 65 and 116 days at 5 µg/kg and 130 to 211 days at 1 µg/kg.

### 7.3  Long-term Toxicity

Chronic toxicity studies performed on laboratory animals exposed to TCDD are summarized in Table 50. Studies on carcinogenicity are presented in section 7.7.

### 7.3.1  Studies on rats

In studies by Van Miller et al. (1977), male Sprague Dawley rats were maintained in groups of 10 on diets containing 0, 1, 5, 50, 500, 1000, 5000, 50 000, 500 000, and 1 000 000 ng TCDD/kg for 65 weeks and survival was monitored. At the five highest dose levels, all animals died before the study was completed. The first deaths in these treated groups occurred by weeks 31, 31, 3, 2, and 2 of treatment, respectively. Groups receiving 50, 500, or 1000 ng TCDD/kg in the diet died from acute toxic effects including severe liver necrosis, bile duct hyperplasia and oedema, atrophy of spleen and thymus, and gastrointestinal haemorrhages.

Groups of 50 male and 50 female Sprague Dawley rats were fed diets providing daily doses of 0.001, 0.01, and 0.1 µg TCDD/kg body weight for 2 years (Kociba et al., 1978, 1979a,b). Control rats, 86 males and 86 females, received diets to which the vehicle acetone had been added. The dose levels corresponded to a dietary content of 22, 208, and 2193 ng TCDD/kg feed. Increased mortality was observed in females given 0.1 µg/kg per day, while no increased mortality was observed in male rats at this dose or in animals receiving doses of

0.01 or 0.001 µg/kg per day. From month 6 to the end of the study the mean body weights of males and females decreased at the highest dose and to a lesser degree in females given 0.01 µg/kg per day. During the course of the study, subnormal body weights were occasionally also recorded in the low-dose group, although during the last quarter of the study the body weights were similar to those of the controls.

Increased urinary coproporphyrin and uroporphyrin were noted in females, but not in males, given TCDD at a dose rate of 0.01 and 0.1 µg/kg per day. Analyses of blood serum collected at terminal necropsy revealed increased enzyme activities related to impaired liver function in female rats given 0.1 µg TCDD/kg per day. Necropsy examination of the rats surviving TCDD exposure to the end of the study revealed that liver effects constituted the most consistent alteration in both males and females. Histopathological examination revealed multiple degenerative inflammatory and necrotic changes in the liver that were more extensive in females. Multinucleated hepatocytes and bile duct hyperplasia were also noted. Liver damage was dose-related and no effect was observable at the lowest dose studied.

7.3.2  Studies on mice

Weekly oral doses of 0, 0.007, 0.7, and 7.0 µg TCDD/kg body weight for 1 year resulted in amyloidosis and dermatitis in male Swiss

mice (Toth et al., 1979). The incidence of these lesions was 0/38, 5/44, 10/44, and 17/43 in the control, low-, medium-, and high-dose groups, respectively.

7.3.3  Studies on monkeys

     In a study by Allen & Carstens (1967) groups of four to five rhesus monkeys were fed diets containing 0, 0.125, 0.25, 0.5, 1.0, and 10.0% of fat (which had been shown to be toxic to chickens) until death. The "toxic fat" was later demonstrated to contain various PCDDs, of which 65% by mass of the total PCDDs present was TCDD (Norback & Allen, 1973). The survival time became shorter with increasing doses of "toxic fat". Mean time to death was 445 days for the low dose and 91 days for the high dose. Decreased food consumption and progressive body weight loss, compared with controls, were noted. Both clinical and histological changes near the time of death appeared similar regardless of dose. The monkeys developed subcutaneous oedema, progressing from the eyelids and face, ascites, and hydropericardium. Characteristic skin changes were observed as well as anaemia, leukopenia, and hypoproteinaemia. The bone marrow was hypoplastic. Centrilobular necrosis, bile duct hyperplasia, and multinucleated hepatocytes were found in the liver. In more than half of the animals, there was marked hypertrophy of the gastric mucosa, with crypts and mucin-containing cysts penetrating into the submucosa. Ulcerations in the fundic and pyloric regions were observed.

     Allen et al. (1977) fed eight adult female rhesus monkeys a diet containing 500 ng TCDD/kg for 9 months. There after, surviving animals were removed from the TCDD diet and were observed for another 4 months. No control animals were included, and so data were compared with pre-exposure values where possible. During the first 3 months of exposure animals developed periorbital oedema, acne, and loss of facial hair and eyelashes. By 6 months these changes were quite prominent in six out of eight monkeys, and a decrease in haemoglobin haematocrit was noticed. The animals lost weight even though their food intake was unaltered. Two animals died within the 9-month exposure period and three monkeys continued to develop toxic symptoms and died after 3 months on a TCDD-free diet. The three surviving animals continued to experience periorbital oedema and loss of hair. The total intake of TCDD over the 9-month period was calculated to be 2-3 μg/kg body weight. Death was preceded by severe anaemia, a decreased white blood cell count, and severe thrombocytopenia. Autopsy findings included haemorrhage into a variety of organs, ascites, and subcutaneous oedema. Hypertrophy, dilatation, oedema, and hydropic degeneration of the myocardium were noted in all animals. The biliary ducts showed marked dilatation. Moderate hyperkeratosis of the skin,

with cystic keratosis of the hair follicles, was noted, and
hypocellularity of the lymphoid tissue and the bone marrow were
observed. The hyperplastic mucous-secreting cells of the gastric
epithelium had invaded the submucosa, and ulceration and mucinous
cysts were common in the modified gastric mucosa. Hypertrophy and
hyperplasia of the epithelial lining of the biliary system were
present. The bronchial epithelium, salivary glands, bile ducts, and
pancreatic ducts showed metaplastic changes. Death was attributed to
complications from the severe pancytopenia. The same pattern of
morphological changes were reported to occur in a similar study
performed by Barsotti et al. (1979).

Similar, though less severe, effects were observed in four adult
female rhesus monkeys fed a diet of 50 ng TCDD/kg for 20 months
(Schantz et al., 1978).

## 7.4  Effects Detected By Special Studies

### 7.4.1  Wasting syndrome

TCDD causes a starvation-like or wasting syndrome in several
animal species. In young animals, or following a sublethal dose in
adults, this response is manifested as a cessation of weight gain.
Early studies suggested that acute or chronic treatment with TCDD
decreased food consumption, but insufficiently to account for the
weight loss (Allen et al., 1975, 1977; Greig et al., 1973; Harris et
al., 1973; Kociba et al., 1976; McConnell et al., 1978a,b). To
elucidate whether malabsorption could explain the wasting syndrome,
the transfer of a number of nutrients has been studied with everted
intestinal sacs from TCDD-treated rats. A transient increase in the

serosal transfer of $^{59}$Fe in Sprague Dawley rats was reported by
Manis & Kim (1979). Absorption of glucose (Ball & Chabra, 1981; Madge,
1977) and lipids (Shoaf & Shiller, 1981) was decreased by TCDD
treatment. The absorption of cobalt, galactose, and proline (Manis &
Kim, 1977) as well as of D-galactose, L-arginine, L-histidine (Madge,
1977), and penicillin (Manis & Apap, 1979) was reported to be
unaffected by TCDD treatment. Leucine transport was depressed in
Sprague Dawley rats 4 h after a single oral dose of 100 µg TCDD/kg
body weight (Ball & Chabra, 1981), whereas no effect was observed in
Fisher rats 7 days after exposure to 80 µg TCDD/kg body weight
(Schiller et al., 1982). Neal et al. (1979) found normal absorption
and intermediary metabolism of glucose, L-alanine, and oleate in
guinea-pigs treated with a single oral dose of 2 µg TCDD/kg body
weight. Apparently there was no generalized impairment of intestinal
absorption. The effects reported may well be secondary to decreased

food consumption which by itself causes structural changes in the intestine (Steiner et al., 1968) as well as impaired absorption of nutrients (Esposito et al., 1967).

The connection between the wasting syndrome and the lethal effect of TCDD has been investigated in pair-feeding and forced nutrition studies. Courtney et al. (1978) fed TCDD- treated female Wistar rats a normal pelleted diet ad libitum. Supplementation with water, electrolyte solution, or liquid diet, administered by gavage, could not reverse or change the pattern or extent of TCDD-induced weight loss or mortality.

To bypass gastrointestinal absorption, Gasiewicz et al. (1980) fed rats intravenously with total parenteral nutrition (TPN). Rats that had received a single ip dose of 100 µg TCDD/kg body weight gained weight similarly to their TPN-fed controls, yet still died at days 13 to 17 following treatment. TCDD-treated rats fed a chow diet ad libitum lost weight progressively (as compared to pair-fed controls that maintained their starting weight) and died at days 11 to 20. In TPN-fed TCDD-treated rats, liver damage was more severe and fat depots were increased as compared to chow-fed TCDD-treated animals. Similar results were obtained with TPN-fed male Hartley guinea-pigs treated with a single ip dose of 2 µg TCDD/kg body weight in olive oil, when compared to TPN-fed control guinea-pigs (Lu et al., 1986). Similar signs of toxicity were present in TPN- and ad libitum-fed TCDD-treated guinea-pigs. In contrast to TPN-fed rats (Gasiewicz et al., 1980), TCDD- treatment in TPN-fed guinea-pigs only produced mild hepatic changes, including increased liver lipid and cytochrome P 450 content, but no morphological changes (Lu et al., 1986).

Seefeld et al. (1984a) suggested that TPN-fed TCDD- treated rats might have suffered from overnutrition and, secondary to that, enhanced hepatotoxicity, as compared to chow-fed, TCDD-treated rats. These same investigators have presented a heuristic model for the

TCDD-induced wasting syndrome based on the assumption that body weight in rats is regulated around an internal standard or set point (Keesey et al., 1976). Prevailing weight at a given age is constantly being compared to this set point value and if differences occur, feed consumption is adjusted so as to raise or lower body weight to match the set point level. If TCDD lowers this setpoint, reduction in food consumption would result, as the rat attempts to reduce its weight to a new lower level of regulation determined by the dose of TCDD administered. This hypothesis has been tested in several experiments under carefully controlled feeding procedures.

Repeated studies have demonstrated that reduction of feed intake, due to increased food spillage, is sufficient to account for the loss of body weight in TCDD-treated Sprague- Dawley rats (Seefeld & Peterson, 1983, 1984; Seefeld et al., 1984a,b). TCDD-treated rats maintain and defend their reduced weight level with the same precision as control rats (fed ad libitum) defend their normal weight level (Seefeld et al., 1984b). The percentage of the daily feed intake that is absorbed by the gastrointestinal tract of TCDD-treated and control rats is similar (Potter et al., 1986a; Seefeld & Peterson, 1984). Water intake, resting and total oxygen consumption, carbon dioxide production, respiratory quotient, and spontaneous motoractivity were decreased in a dose-dependent manner by TCDD-treatment (Potter et al., 1986a; Seefeld & Peterson, 1983; Seefeld et al., 1984a). Urine output was unaffected by TCDD-treatment, despite decreased water intake, whereas urinary excretion of energy and urea were decreased and urinary ammonia was increased (Potter et al., 1986a).

Hypophagia was the major cause of the loss of adipose and lean tissue in male Fisher F-344 rats, C57Bl/6 mice, and albino guinea-pigs when exposed to a calculated LD80 dose of TCDD (Kelling et al., 1985). Body weight loss followed a similar time course in TCDD-treated and pair-fed control animals of all three species. Lethalities for TCDD-exposed rats, mice, and guinea-pigs were 95%, 69%, and 81%, respectively, compared to lethalities in the appropriate pair-fed controls of 48, 14, and 64%, respectively (Kelling et al., 1985). Lethality and body weight loss followed almost identical time-course-curves in Sprague Dawley rats that received a single oral dose of 75 µg TCDD/kg body weight and in pair-fed controls (Christian et al., 1986a). Thus the contribution to lethality made by body weight loss seems to depend on the species and strain. Weight loss appears to play a greater role in causing death in Sprague Dawley rats and guinea-pigs than in Fisher F-344 rats and C57Bl/6 mice. Christian et al. (1986a) demonstrated differences in organ weights and histopathology in TCDD-treated and pair-fed animals, despite similar time-courses and magnitude of body weight loss and lethality. Pair-fed animals exhibited lesions in the gastro-intestinal tract, which were absent in TCDD-treated rats, that may have contributed to death. The hepatic carbohydrate, protein, and lipid metabolism was affected

differently in TCDD-treated and pair-fed Sprague Dawley rats (Christian et al., 1986b). To distinguish direct effects of TCDD from effects secondary to hypophagia, the studies by Christian et al. (1986a,b) and Potter et al. (1986a) were performed with schedule-fed animals. The reason for this was the finding that the 24-h feeding pattern for TCDD-treated rats was different from the feeding pattern for pair-fed controls, although it was similar to that of control rats

fed ad libitum. Decreased feed consumption did not contribute to weight loss in C57BL/6 mice exposed to TCDD until the animals were moribund (Chapman & Schiller, 1985).

Besides being typical signs of TCDD toxicity, loss of body weight and appetite are also prominent signs of thyroid dysfunction (see section 8.4.9). Serum glucose levels were also decreased by TCDD independently of hypophagia, whereas the decrease in serum insulin appeared to result from hypophagia, since it was seen in both TCDD-treated and pair-fed controls. These results indicate that the effect of TCDD on thyroid hormones cannot explain the TCDD-induced decrease in body weight gain.

An interesting biochemical effect in TCDD-induced wasting is the ability of TCDD to decrease hepatic vitamin A storage in rats (see section 8.4.10). It has long been known that vitamin A is necessary for growth and that vitamin A deficiency will result in depressed body weight gain as well as in reduced food intake. However, the animal continues to eat and grow though body weight gain is less than normal (Brown & Morgan, 1948; Coward, 1947; Hayes, 1971; Orr & Richards, 1934; Patterson et al., 1942).

The effect of chemical structure on the ability of several PCDDs to cause body weight loss in rats has been investigated (Mason et al., 1986). Of the congeners studied, 2,3,7,8-TCDD was the most active. Those congeners fully substituted in the 2,3,7, and 8 positions but containing additional chlorosubstituents in the non-lateral 1,4,6, and 9 positions were less active.

7.4.2  Hepatotoxicity

TCDD produces hepatomegaly, due to hyperplasia and hypertrophy of parenchymal cells, in all species that have been investigated, even at sublethal doses. However, there is considerable variation between species in the extent of this lesion. Other liver lesions are more species specific.

Liver lesions alone cannot explain lethality following TCDD administration, though it may be a contributing factor at least in the rat and rabbit.

The morphological changes in the liver are accompanied by impaired liver function, characterized by liver enzyme leakage, increased microsomal monooxygenase activities, porphyria, impaired plasma membrane function, hyperlipidaemia, and increased regenerative DNA synthesis.

7.4.2.1  Morphological alterations

    In Charles River rats given single oral sublethal doses of TCDD (5 or 25 µg/kg body weight) a dose-related increase was observed 3 days after dosing in the amount of hepatic smooth endoplasmatic reticulum (SER) around the periphery of cells, particularly in the areas around bile canaliculi. The effect progressed by days 6 and 9, when an increased amount of rough endoplasmatic reticulum (RER) was also present. By day 28 these changes had returned essentially to normal levels (Fowler et al., 1973).

    The livers of CD rats given high sublethal doses of TCDD (5.0 mg/kg body weight per week) for six weeks showed transient degenerative changes, followed by megalocytosis, regeneration, and the occurrence of multinucleated giant hepatocytes (Gupta et al., 1973). They also showed that the hepatotoxic reaction in rats given lethal doses of TCDD (10 µg/kg body weight per day for 16-31 days) was characterized by degenerative and necrotic changes, with the appearance of mononuclear cell infiltration, multinucleated giant hepatocytes, increased numbers of mitotic figures, and pleomorphism of cord cells. These lesions were considered severe enough to be a contributing factor to death.

    Parenchymal cell necrosis was observed by Greig et al. (1973) in Porton rats 3 weeks after exposure to an $LD_{50}$ dose of TCDD. The necrosis, which was located in the centrilobular zone close to the central vein, became more severe with time.

    Jones & Butler (1974) further investigated the time course of the TCDD-induced liver lesions appearing in the centrilobular zone. They confirmed the transient degenerative and inflammatory lesions previously reported (Greig et al., 1973; Gupta et al., 1973). At the ultrastructural level, consistent changes occurred in the cytoplasm whereas normal nuclear morphology and division were found throughout the study. Two weeks after a single oral dose of 200 µg TCDD/kg body weight to Porton rats, extensive fusion of parenchymal cell plasma membranes in the centrilobular zone was replaced by a diffuse zone with islands of normal membrane occurring at intervals. Normal tight and gap junctions were present in control animals and in periportal areas of the test animals. These findings suggest that the multinucleated cells occurring in TCDD-treated rats might form by coalescence of parenchymal cells. The effect of TCDD on plasma

membranes demonstrates a specific subcellular site of action, which might be involved in the toxic action of TCDD. This lesion was,

however, not observed until 2 weeks after treatment and thus could not explain the immediate effects on, for instance, food intake, body weight gain, and general health.

The time course for liver lesions in male Sprague Dawley rats (200 g) given a single ip dose of 20 µg TCDD/kg body weight was followed for up to 32 weeks by Weber et al. (1983). The lesions became progressively worse up to the 16th week after injection, and thereafter appeared to regress slowly. The lesions were almost identical to those reported previously (Fowler et al., 1973; Jones & Butler, 1974). The histological findings were accompanied by hyperbilirubinaemia, hypercholesterolaemia, hyperproteinaemia, and increased serum glutamicoxaloacetic transaminase and serum glutamic-pyruvic transaminase activities, further indicating decreased liver function (Greig et al., 1973; Zinkl et al., 1973).

Fewer studies of liver lesions produced by TCDD have been made in other species than in the rat.

In C57BL/6 mice given single oral doses of 100, 150, or 200 µg TCDD/kg body weight (Vos et al., 1974) and 250 µg TCDD/kg body weight (Jones & Greig, 1975), centrilobular degenerative and necrotic changes were present but multinucleated parenchymal cells were not seen. Proliferation of the bile ducts and bile duct epithelial cells, as well as lipid accumulation, have been observed, with a substantial increase in the hepatic levels of esterified fatty acids and cholesterol. Only slight damage, hepatocellular swelling, was reported in CD-1 mice 21 days after a single dose of 50 µg TCDD/kg body weight, and no histological changes were detected 7 or 35 days after administration (Gupta et al., 1973).

The guinea-pig, while being very sensitive to TCDD, as indicated by $LD_{50}$ data, shows less severe morphological alterations in the liver than do other species. No manifest liver lesions at the light microscopical or ultrastructural levels has been found (Gupta et al., 1973; McConnell et al., 1978b; Moore et al., 1979; Turner & Collins, 1983).

The hamster is very resistant to TCDD toxicity (Table 47) and exhibits no manifest liver damage even after a fatal dose (Henck et al., 1981; Olson et al., 1980b). However, Gasiewicz et al. (1986) found bile duct hyperplasia, numerous inflammatory cells, and increased number of multinucleated cells in the livers of male Golden Syrian hamsters 35 days after they received a single ip dose of 500 µg TCDD/kg body weight in olive oil.

## 7.4.2.2  Hepatic plasma membrane function

The morphological impairment of hepatic plasma membranes in the centrilobular parenchymal cells of TCDD-treated Porton rats was demonstrated histochemically to be preceeded by a loss of ATPase activity (Jones, 1975). The effect occurred 3 days after treatment in an area five to six cells deep around the central vein along the canalicular borders, and became more severe with time. At the end of the study (day 42 posttreatment) the ATPase activity was completely abolished around the central vein, including the mid-zonal region, and encroached on the periportal area in moribund animals. The loss of ATPase activity was related to the clinical state of the animal. Thus animals displaying minimal signs of intoxication retained the normal distribution of ATPase in the periportal zone. In animals killed 9 months after treatment, partial restoration of the normal liver architecture and the ATPase activity were evident.

Biochemical studies of isolated heptatic plasma membranes from Holtzman rats treated with 10 or 25 µg TCDD/kg body weight revealed depressed ATPase activities (Peterson et al., 1979a). The activity of Na/K-ATPase was depressed to the same extent for both doses from day 2 to 40 after treatment, while a similar depression of Mg-ATPase activity was observed only in the high-dose group. The Mg-ATPase activity tended to recover by day 40, whereas Na/K-ATPase activities did not. A pair-feeding experiment demonstrated that these effects were independent of the TCDD-induced decrease in food consumption. In vitro incubation of plasma membranes indicated that ATPase inhibition did not occur by direct interaction with TCDD. Greig & Osborne (1981) demonstrated a decrease in K/Mg-ATPase activity, but not in the Na/K-ATPase or 5'-nucleotidase activities of hepatic plasma membranes prepared from female Porton rats 6 and 11 days after a dose of 200 µg TCDD/kg body weight.

Many physiological homeostatic mechanisms are dependent on proper plasma membrane function and composition. Matsumura et al. (1984) reported that a single ip dose of 25 µg TCDD/kg body weight to male Sprague Dawley rats had reduced hepatic plasma membrane ATPase-activities by 40% 10 days after treatment. The marker enzyme for putative preneoplastic hepatocytes, glutamyl transpeptidase, was reduced, while protein kinase (both c-AMP-stimulated and c-AMP-nonstimulated) was increased (Matsumura et al., 1984). Both c-AMP-dependent and c-AMP-independent protein kinase, in hepatic plasma membranes from male Sprague Dawley rats treated with an ip dose of 25 µg TCDD/kg body weight, were maximally increased on day 20 after treatment (4.5- and 12-fold, respectively). The induction was measurable within 2 days after the administration and was still

persistent after 40 days (Bombick et al 1985). Protein kinase C was significantly increased in hepatic plasma membranes from Sprague Dawley rats but not from guinea-pigs 10 days after single ip doses of 25 and 1 µg TCDD/kg body weight, respectively (Bombick et al., 1985). TCDD treatment in vivo also affected the in vitro binding of

concanavalin A, epidermal growth factor, and insulin to their cell surface membrane receptors.

The binding of glucagon and prostaglandin E were not affected by a dose of 25 µg TCDD/kg body weight (Matsumura et al., 1984). Studies with Sprague Dawley rats demonstrated that the TCDD-induced decrease in epidermal growth factor binding (EGF) was observable within 2 days after dosing, reached its maximum by day 20, and was still significant 40 days after dosing. The decrease was observable after a single ip dose of 0.1 µg TCDD/kg body weight (Madhukar et al., 1984). The relative doses of TCDD needed to suppress EGF binding to 50% of the control level were 1, 14, and 32 µg/kg body weight for the guinea-pig, the Sprague Dawley rat, and the Golden Syrian hamster, respectively (Madhukar et al., 1984). A single ip dose of 115 µg TCDD/kg body weight had decreased the EGF binding 10 days after treatment by 93.1, 97.8, and 46.0% in C57Bl/6, CBA, and AKR mice, respectively (Madhukar et al., 1984). The effect of daily sc injections of 2 ng EGF/kg body weight to newborn Balb/c-mice was compared with the effect of a single ip dose of 10 mg TCDD/kg body weight given to the dam of newborn Balb/c-mice within 3 h of delivery (Madhukar et al., 1984). The parameters studied, all well known in vivo effects of EGF, were: time for eyelid opening and tooth eruption; hair length and diameter on day 14; and body weight and thymus weight on day 22. All parameters were significantly reduced both by TCDD treatment and EGF treatment, as compared to controls.

Bombick et al. (1984) found that, 10 days after treatment, in vitro binding of [125]I-low density lipoprotein (LDL) to its receptor on hepatic plasma membranes was decreased by 73% in TCDD-treated guinea-pigs (1 µg TCDD/kg body weight) as compared to pair-fed controls. Primary hepatocytes from guinea-pigs treated in the same way had a reduced ability to internalize [125]I-LDL. The reduction of LDL receptors on hepatic plasma membranes might be responsible for the increase in plasma of very low density lipoproteins (VLDL) and LDL noted in TCDD-treated animals.

Quantitative changes in the protein composition of plasma membranes following TCDD treatment have been reported (Brewster et al., 1982; Matsumara et al., 1984). The membranes were isolated from male Sprague Dawley rats 10 days after an ip injection of 25 µg

TCDD/kg body weight and analyzed by SDS-polyacrylamide gel
electrophoresis. Some small proteins (14 000-30 000 daltons) were
completely abolished by TCDD treatment. The effect was most pronounced
10 to 20 days after treatment.

7.4.2.3  Biliary excretion

     The early proliferation of liver cells around bile canaliculi
seen after TCDD treatment (Fowler et al., 1973) was suggestive of an
effect on biliary excretion.

     The cumulative biliary excretion of indocyanine green (ICG), an
organic anion, was decreased in a dose-dependent manner by treatment
with 5 or 25 µg TCDD/kg body weight in CD rats (Hwang, 1973). On the
contrary, biliary excretion of the organic anions sulfobromophthalein
and phenol-3,6-dibromophthalein was unaffected by treatment with 10 or
25 µg TCDD/kg body weight in Holtzman rats (Yang et al., 1977).

     Biliary excretion of ouabain, a model compound for neutral
non-metabolized substrates such as estradiol, progesterone, and
cortisol, was depressed in male Holtzman rats in a dose-related manner
by a single oral dose of 10 or 25 µg TCDD/kg body weight (Yang et al.,
1977). The effect was detectable two days after treatment, reached a
peak between 10 and 20 days, and recovery was only slight by day 40.
Increased plasma concentration and decreased bilary excretion of
ouabain was also measured in male Sprague Dawley rats 10 days after a
single oral dose (25 µg/kg body weight) of TCDD, or
1,2,3,7,8,9-hexaCDD (Yang et al., 1983b). Two other congeners,
1,2,4,6,7,9-hexaCDD and 1,3,6,8-tetraCDD had no effect on these
parameters.

     When hepatocytes from TCDD-treated (10 µg/kg body weight) male
Sprague Dawley rats were incubated with labelled ouabain or procaine
amide ethobromide (PAEB) 10 days post-treatment, both the rate of
uptake and the steady-state concentration of ouabain were decreased,
whereas the uptake of PAEB was unaffected by TCDD (Eaton & Klaassen,
1979). The dose of TCDD was very small relative to ouabain
(approximately 150 µmol/litre), so it is not likely that TCDD exerted
its effect by competing with the drug for transport into bile. These
data suggest that the hepatic membrane transport process for ouabain
may be selectively damaged by TCDD.

     Peterson et al. (1979a) observed a positive correlation between
the levels of hepatic plasma membrane ATPase activities, biliary
excretion of ouabain, and bile flow in vivo after TCDD treatment.
However, in a further experiment, using perfused rat liver, Peterson

et al. (1979b) demonstrated that biliary excretion of ouabain and liver membrane ATPase activities could be decreased independently. Therefore, ATPase activities cannot be directly responsible for the reduced ouabain excretion.

7.4.3  Porphyria

     Chronic sublethal exposure to TCDD produces an accumulation of porphyrins in the liver and an increase in urinary porphyrin excretion. In stages of manifest porphyria, accumulation of porphyrins occurs not only in the liver but also in the kidney and spleen (Goldstein et al., 1982). It was demonstrated that mice respond to four weekly doses of 25 µg TCDD/kg body weight with hepatic porphyria, accompanied by an increase in aminolevulinic acid (ALA) synthetase activity, and liver lesions (Goldstein et al., 1973), whereas a single

dose of 5, 25, or 100 µg TCDD/kg body weight did not induce porphyria nor ALA synthetase activity in the rat (Woods, 1973). The suggested species difference was later ruled out by Cantoni et al. (1981) and Goldstein et al. (1976, 1982). Chronic administration of 1 µg TCDD/kg body weight per week to rats for 16 weeks resulted in hepatic porphyria (Goldstein, 1976a,b, 1982). In contrast, single oral doses as high as 30 µg TCDD/kg did not produce porphyria either acutely or 16 weeks later. A 6-month recovery period following the final dose was not long enough to reverse the porphyria. Urinary porphyrins and hepatic ALA synthetase activity remained maximally elevated, while hepatic porphyrin levels decreased during this period. Failure to demonstrate porphyria in rats after chronic administration of TCDD for 13 weeks (Kociba et al., 1976) or 2 years (Kociba et al., 1978) was suggested to be the result of unsatisfactory porphyrin analysis (Goldstein et al., 1982).

     To further characterize TCDD-induced porphyria, Cantoni et al. (1981) performed a 45-week study to follow the pattern of porphyrin excretion in rats exposed orally to 0.01, 0.1, or 1.0 µg TCDD/kg body weight/week. They found an increase in the coproporphyrin level in the initial phase of exposure, which remained the only sign of exposure in the lowest-dose group. A marked porphyric state appeared only in the 1.0 µg/kg dose group, commencing 8 months after dosing started. At that time urinary porphyrin excretion was 70 times higher than in control rats. The excretion pattern was characterized by increased levels of carboxylated porphyrins.

     In attempts to elucidate the mechanism of TCDD-induced porphyria, the effects of TCDD on the enzymes involved in the synthesis and catabolism of porphyrins have been studied. TCDD was found to be a

potent inducer of ALA synthetase, the initial and rate-limiting enzyme in haeme synthesis in the liver of chicken embryos (Poland & Glover, 1973b). Elevated ALA synthetase activity has since been demonstrated also in mice and rats (Goldstein et al., 1973, 1982; Kociba et al., 1976, 1978). However, the TCDD-induced increase does not appear after acute exposure and only after several weeks of chronic exposure to TCDD. Jones & Sweeny (1980) failed to demonstrate increased ALA activity in mice exposed to 25 µg TCDD/kg body weight per week for 11 weeks, although porphyria was evident. Thus, induction of ALA synthetase does not seem to be the primary event in TCDD-induced porphyria. Elder et al. (1976, 1978) suggested that decreased hepatic porphyrinogen decarboxylase is the primary event in porphyria induced by halogenated aromatics. TCDD depresses this enzyme activity _in_ vivo in the liver of mice (Jones & Sweeny, 1980; Elder & Sheppard, 1982; Cantoni et al., 1984a,b) but not in vitro (Cantoni et al., 1984b).

A decrease in porphyrinogen decarboxylase activity was present one week after a single dose of 75 µg/kg body weight and continued to decrease with time, thus preceding the increase in hepatic porphyrins, which started to rise during the first 2 weeks after treatment (Smith et al., 1981). TCDD- induced depression of hepatic uroporphyrinogen decarboxylase activity occurs also in the newly regenerating liver, as demonstrated by Smith et al. (1985) in C57Bl/10 mice 10 days after partial (2/3) hepatectomy. The mice were treated with 75 µg TCDD/kg body weight orally 4 weeks before hepatectomy. Greig et al. (1984) demonstrated that pretreatment of five different strains of mice with 12.5 mg $Fe^{2+}$ one week before the administration of 75 µg TCDD/kg body weight had a synergistic effect on porphyria assessed 5 weeks after dosing, expressed as increased hepatic porphyrin and decreased porphyrinogen decarboxylase activity. Iron alone did not increase hepatic porphyrin levels, nor did it affect hepatic porphyrinogen decarboxylase activity.

### 7.4.4  Epidermal effects

Chloracne and associated pathological changes in the skin are among the most sensitive and widespread responses to TCDD in humans. Similar skin toxicity is expressed only in a limited number of animal species, namely rabbits, monkeys, and hairless mice. To characterize the epidermal response and to elucidate the mechanism(s) of toxicity to epidermal cells, numerous studies have been performed both _in_ vivo and _in vitro._

### 7.4.4.1  In vivo studies

The acnegenic activity of TCDD and related compounds has been tested in the rabbit ear bioassay, first developed by Adams et al. (1941) for industrial applications. The test substance is applied to the inner surface of one of the ears, while pure vehicle is applied to the other. Responses indicative of acnegenic activity include comedo formation, increased ear thickness, and hyperkeratosis. Mild irritation, increased ear thickness, slight enlargement of follicular aperture, slight exfoliation and slight crust formation alone are not considered indicative of acnegenic activity. Microscopically there is conversion of sebaceous cells in the hair follicles into keratin-forming cells. A dose-dependent, positive response was found in this assay when a total dose of 1, 3, or 10 µg TCDD was applied on three successive days (Jones & Krizek, 1962). Also Schwetz et al. (1973) found a dose-dependent acnegenic response in the rabbit-ear bioassay after repeated applications of 4-40 µg TCDD/ear, five days per week for four weeks, corresponding to total doses of 80-800 µg. No response was obtained when the total application was 8 ng. Poiger & Schlatter (1980) applied a single dose of TCDD in various vehicles on the inner surface of the rabbit ear and followed the appearance of inflammation, hyperkeratosis, and chloracne. The minimum dose that induced skin lesions was around 1 µg TCDD/ear when the vehicle was

acetone, vaseline, or polyethylene glycol 1500 with 15% water. When TCDD was mixed with soil-water (2:1), or activated carbon-water (1:8) before application, 2-3 and 160 µg TCDD/ear, respectively, were needed to induce lesions. Even 160 µg TCDD/ear produced very small changes on the skin surface when applied as an activated carbon-water paste.

Hairless mice constitute another _in vivo_ model for studies of epidermal effects of TCDD. Puhvel et al. (1982) studied cutaneous changes induced by topical application of 0.1 µg TCDD, three times per week for four weeks, in two strains of hairless mice (Skh:HR-1 and HRS/J). Epidermal hyperplasia, hyperkeratinization, loss of sebaceous glands and follicles, and keratin build-up in the dermal cysts were noted in both strains of mice. Follicular keratosis, considered the pathognomonic lesion in human chloracne, did not appear within 4 weeks of application. In the same study, follicular keratosis did develop after topical application of 2 mg 3,4,3',4'- tetrachlorobiphenyl, five times per week for 8 weeks, suggesting that follicular keratosis is an extension of the epidermal response and, thus, not related to metabolic changes in sebaceous glands. The authors considered hairless mice a less sensitive model for the chloracnegenic response than the rabbit ear bioassay. Similar findings were obtained when HRS/J mice were exposed to TCDD topically applied two to three times per week for four weeks (Knutson & Poland, 1982; Poland & Knutson, 1982). They found, with a total applied dose of 1.2 µg TCDD, a moderate to severe

response, including hyperplasia, hyperkeratosis of the interfollicular epidermis, squamous metaplasia of the sebaceous glands, and hyperkeratosis within the dermal cysts, but no keratosis in the sebaceous follicles.

Soot or a benzene extract of soot, containing PCDDs and PCDFs from a PCB-containing transformer fire (Binghamton, New York, USA), were applied to 64.5 cm$^2$ of the shaved, unabraded dorsal surface of (3 + 3) and (1 + 1) male and female New Zealand white rabbits (3.5 kg), respectively (Silkworth et al., 1982). The dose applied was 500 mg soot or benzene extract corresponding to 500 mg soot/kg body weight. Controls, one male plus one female, were exposed to activated carbon or benzene in corresponding amounts. Exposure lasted for 24 h and the observation period was 67 days. The soot produced no overt toxicity, no weight loss, and no histological findings in thymus, kidney, or skin, but hepatic centrilobular hypertrophy was found in both sexes. The soot extract gave rise to a reversible skin inflammation and hepatic centrilobular hypertrophy in the female only. No weight loss was recorded and the kidney, thymus, and skin were histologically normal at necropsy.

7.4.4.2  In vitro studies

Keratinocytes, the principal cell type of epidermis, form an _in vitro_ model for studies of TCDD-induced hyperkeratosis both in human and animal-derived cell cultures. The response is analogous to hyperkeratinization _in vivo_. Newly confluent epidermal cell cultures

exhibit proliferative properties, while the number of basal cells tends to decrease with increasing time of post-confluency growth. Thus, with the appropriate selection of culture medium and time of treatment, different aspects of TCDD toxicity _in vivo_ can be modelled _in vitro_.

A TCDD-induced response of _in vitro_ keratinization was first demonstrated in XB cell cultures, an established keratinocyte cell line derived from a mouse teratoma, plated at high density to avoid spontaneous keratinization (Knutson & Poland, 1980b). Keratinization was dose-related and histologically similar to that which occurs spontaneously when XB cells are plated at low density. The epidermal proliferation in XB cells produced by TCDD could not be biochemically related to the response produced by cholera toxin, epidermal growth factor, or 12-_O_-tetradecanoylphorbol-13-acetate, other compounds known to affect cell proliferation in XB cells (Knutson & Poland, 1984). Late passage XB cells, i.e. XBF cells, show increased cell density at saturation and a fusiform morphology at high density.

Additionally, they have lost their ability to respond with keratinization upon TCDD treatment. Exposing XBF cells to TCDD concentrations in the range 10 to 11 X $10^{-8}$ mol/litre resulted in normal growth until confluency was reached by day 7. Thereafter TCDD-treated cultures showed a persistent decrease in cell growth and cell proliferation as well as changed morphology, whereas viability was unaffected (Gierthy & Crane, 1984). Reseeding these quiescent XBF cells, previously exposed to $10^{-9}$ mol/litre TCDD for 14 days, resulted in normal growth and proliferation until confluency. These TCDD-pretreated cells maintained their susceptibility to TCDD-induced changes in cell growth and morphology. Both XB cells (keratinization assay) and XBF cells (flat-cell-assay) have proved to be useful in vitro bioassays to measure "dioxin-like" activity of both environmental samples and of pure isomers (Gierthy et al., 1984; Gierthy & Crane, 1985a,b). Although XBF cells, a highly transformed variant of XB cells, seem to be less appropriate as a model for TCDD action on normal mammalian epithelial cell proliferation and differentiation, they seem to be more stable and easier to maintain than XB cells. Several continuous lines of human keratinocytes derived from neonatal foreskin (Milstone & Lavigne, 1984; Osborne et al., 1984) or squamous cell carcinomas (SCC) (Rice & Cline, 1984; Willey et al., 1984; Hudson et al., 1985, 1986) have been shown to respond to TCDD in nanomolar concentrations with a variety of signs that indicate alterations in the normal differentiation process. Stimulation of $^{3}$H-thymidine incorporation was seen in post-confluent human epidermal cells derived from neonatal foreskin after exposure to TCDD (Milstone & Lavigne, 1984). Newly confluent human epidermal cells, derived from foreskin, responded to exposure for 4 days to 10 nmol TCDD/litre with decreased DNA synthesis, a decrease in the number of proliferating basal cells, decreased binding of epidermal growth factor (EGF), an increase in the number of differentiated cells, and increased envelope formation, i.e., a decrease in the proliferative

capacity and an increase in the state of differentiation (Osborne & Greenlee, 1985). The decreases in small (basal) cell number and EGF binding were dose dependent, with EC50 values for TCDD of 2 and 1 nmol/litre, respectively. The responses were also obtained with TCDF but not with 2,4-diCDD.

The proliferation and differentiation of epidermal cells is normally regulated by several growth factors and hormones, e.g., EGF, vitamin A and hydrocortisone. Mouse hepatoma cells exposed to TCDD for 24 h showed 20% inhibition of EGF binding (Karenlampi et al., 1983).

Hudson et al. (1985, 1986) demonstrated that TCDD decreases, in a dose-dependent manner, the specific binding as well as the cellular

uptake of EGF in cultures of human epidermal cells. The $EC_{50}$ dose for inhibition was 1.8 nmol/litre. A similar inhibitory effect was obtained by TCDF, while 2,7-diCDD was inactive even at doses 100-fold greater. Maximal inhibition, almost 60%, of EGF binding in confluent SCC-12F cells exposed to 100 nmol TCDD/litre was obtained after a pretreatment period of 72 h. No effect was obtained when TCDD was added at the same time as EGF; thus TCDD did not compete for EGF-binding sites, neither did TCDD affect the process of internalizing the EGF. Further studies of the SCC-12F cell line revealed data suggesting that TCDD specifically reduces the high affinity EGF-binding sites in the basal cell population of this cell line (Hudson et al., 1986).

The addition of $10^{-6}$ mol hydrocortisone/litre to the medium antagonized the growth inhibition of SCC cells grown in $10^{-10}$ mol TCDD/litre (Rice & Cline, 1984). Hydrocortisone stimulated several aspects of keratinocyte differentiation. These stimulatory effects were abolished in the presence of $10^{-8}$ mol TCDD/litre, although TCDD alone had no effect on these parameters. The hydrocortisone level in the medium was unaffected by TCDD. TCDD, and even more so hydrocortisone, were able to stimulate stratification in SCC cultures held at confluence for extended periods. This effect was opposed by vitamin A (Rice & Cline, 1984).

Like TCDD, vitamin A suppressed the stimulation of keratinocyte differentiation by hydrocortisone. However, vitamin A had no effect on TCDD-induced growth inhibition or its reversal by hydrocortisone (Rice et al., 1983; Rice & Cline, 1984).

Epidermal transglutaminase (ETG) activity, the marker enzyme for terminal differentiation, was increased by treatment of basal keratinocyte cultures from neonatal BALB mice with $10^{-9}$ mol TCDD/litre for 5 to 12 days, although morphologically no signs of terminal differentiation were present. A parallel increase in ETG activity was present when these cells were grown in medium rich in $Ca^{2+}$, although these cells did stratify and differentiate (Puhvel et al., 1984).

7.4.5  Effects on the immune system

TCDD produces a pronounced atrophy of the thymus, spleen, and, to a lesser, extent the peripheral lymph nodes of most experimental animals. Since Buu-Hoi et al. (1972a) reported on TCDD-induced thymic atrophy, many studies in rats, mice, guinea-pigs, and monkeys have shown that the thymus is one of the organs most severely affected by

TCDD. Lesions in the thymus appear at exposure levels well below those inducing lesions in other organs. Although there is species variation in the degree and severity of other organ effects, the effects of TCDD on lymphoid tissues is consistent in all species. Further investigations of the effect of TCDD on the immune system, which is a rapidly proliferating and differentiating organ system containing many cellular components in a highly organized and regulated network, have revealed that TCDD affects both the humoral-mediated (section 7.4.5.2) and the cell-mediated immune response (section 7.4.5.3). Also the complement system, a key component of the innate immunity, is affected by TCDD treatment (White et al., 1986). Damage to the thymus and to the cell-mediated immune system seems to be rather specific in that it occurs at doses considerably lower than those affecting other immune functions (Faith & Luster, 1979). Thymic involution is believed to be a direct effect on the gland, and not secondary to factors such as undernutrition (van Logten et al., 1980), altered levels of hormones including corticosteroids (Vos et al., 1973; van Logten et al., 1980), pituitary hormones (Vos et al., 1973), and thymosin (Vos et al., 1978a,b), or zinc deficiency (Vos et al., 1978a,b), or to a direct cytotoxic effect on lymphocytes (Vos et al., 1978a,b). A direct effect of TCDD on mouse fetal thymus organ cells grown in vitro was demonstrated at concentrations as low as $10^{-10}$ mol/litre (Dencker et al., 1985). Within the thymus, lymphocytes in the cortex, i.e., the immature T-cells, are more severely affected in TCDD-treated animals than are lymphocytes in the medulla, i.e., the mature T-cells. Thus, it seems that TCDD impairs the differentiation of thymocytes into immunocompetent T cells. Greenlee et al., (1985) obtained results demonstrating a direct effect of TCDD on thymus epithelial (TE) cells.

High levels of Ah receptors (section 7.8.1) have been found in the thymus (Carlstedt-Duke, 1979; Mason & Okey, 1982; Gasiewicz & Rucci, 1984). Studies with C57Bl/6 mice (responsive to TCDD), DBA/2 mice (less responsive to TCDD), and B6D2F1 mice, hybrid mice from crosses between these strains, suggest that TCDD-induced thymic involution, as well as immunosuppression, segregates with the Ah locus in these strains of mice (Poland & Glover, 1980; Clark et al., 1983; Vecchi et al., 1983; Nagarkatti et al., 1984; Dencker et al., 1985). The most profound and persistent effect of TCDD on the immune system is found when TCDD is administered during pre- and/or immediate postnatal life. Contrary to other experimental animals investigated, rainbow trout (Salmo gairdneri) are relatively resistant to the immunosuppressive effects of TCDD (Spitzbergen et al., 1986). No humoral-mediated effects, and only minor cell-mediated effects, were present at doses which caused clinical toxicity.

7.4.5.1  Histopathology

Lymphoid organs, primarily thymus but also spleen and lymph nodes, have been found to be affected by TCDD over a wide spectrum of dose ranges in adult rats, guinea-pigs, and mice (Gupta et al., 1973; Vos et al., 1973; Vos and Moore, 1974; McConnell et al., 1978b). The marked reduction in the size of thymus has been referred to as atrophy (Gupta et al., 1973; Vos & Moore, 1974), regression (Allen et al., 1975), or involution (Kociba et al., 1976), though these terms do not clearly represent the pathogenesis of this lesion but are more a description of the final event. Toxic effects on thymus appeared in adult guinea-pigs, rats, and mice exposed to eight weekly doses of 0.2 µg TCDD/kg body weight, six weekly doses of 5 µg TCDD/kg body weight and four weekly doses of 5 µg TCDD/kg body weight respectively (Vos et al., 1973). The thymus from moribund animals, or from animals that died from TCDD exposure, showed a dose-dependent decrease in the number of cortical lymphocytes, markedly smaller thymic lobules, and loss of demarcation between the cortex and medulla. Guinea-pigs, the species most severely affected by TCDD, showed large cystic Hassall bodies, filled with polymorphonuclear leukocytes (Gupta et al., 1973; Vos et al., 1973). Guinea-pigs that received lethal doses of TCDD showed scattered necrosis of lymphocytes in the cortical region with concomitant phagocytosis by macrophages as early as 5 days post-exposure (McConnell et al., 1978b). The effect was more apparent at day 14, and by day 20 it was difficult to differentiate the cortex from the medulla. At day 20 little evidence of necrosis remained, though karyorrhectic debris and prominent phagocytosis indicated that this had occurred. Since the thymus from guinea-pigs surviving the TCDD dose for 30 days was usually normal microscopically (Vos et al., 1973; McConnell et al., 1978b), it seems that thymic necrosis must be an early event in the course of the toxic syndrome. Furthermore, in animals which survive, thymic regeneration seemed to be rapid. Decreased weight of thymus, loss of cortical cells, and cell necrosis have also been found in hamsters exposed to TCDD (Gasiewicz et al., 1986).

Depletion of lymphoid cells in the spleen, intestinal tract, and various lymph nodes observed in guinea-pigs, rats, and mice (Gupta et al., 1973; McConnell et al., 1978b) was less extensive than in the thymus. The major effect in the spleen of rats is the loss of the T-cell-dependent areas, namely the periarterial lymphoid sheet and the paracortical areas (Vos & Moore, 1974).

Depressed immunoglobulin levels were reported for 1- and 4-month-old C57Bl/6 mice exposed to four and six weekly doses, respectively, of 25 µg TCDD/kg body weight (Vos & Moore, 1974). Feeding 10, 20, 50, or 100 µg TCDD/kg in the diet depressed

dose-dependently the Y-globulin level in 7-week-old Swiss-Webster mice (Hinsdill et al., 1980).

Vos & Moore (1974) demonstrated a dose-related lymphocyte depletion of thymus cortex, spleen, and intestinal lymph nodes in maternally exposed pups of rats and mice. However, no effect on immunoglobulin levels was observed in 25-day-old rats maternally exposed (5 µg TCDD/kg body weight) on days 0, 7, and 14. The developing lymphoid tissues were found to be more sensitive to TCDD than were the lymphoid tissues of adults or young.

7.4.5.2  Humoral-mediated immunity

The humoral-mediated immunity (Tables 51 and 52) operates through antibody-producing cells and is transferable by serum. This system includes classical antibody-mediated protective immunity and immediate hypersensitivity reactions. Vos et al. (1974) reported a significant decrease in the alpha-, ß-, and gamma-globulin levels in C57BL/6 mice given non-toxic doses of TCDD. The effects of TCDD on specific humoral immunity responses in adult animals are summarized in Table 51. Feeding levels of 10 µg TCDD/kg body weight or more reduced the primary and secondary antibody response to both sheep red blood cells (sRBC) and tetanus toxin in male Swiss-Webster mice (Hinsdill et al., 1980). Weekly oral doses of 1 or 10 µg TCDD/kg body weight reduced both the primary and secondary serum antitetanus titres in male New Zealand rabbits (Sharma et al., 1984). The secondary, but not the primary, serum tetanus antitoxin level was decreased in Hartley guinea-pigs given eight weekly doses of 0.2 µg TCDD/kg body weight (Vos et al., 1973). Results presented by Vecchi et al. (1980, 1983) show that single doses as low as 1.2 µg TCDD/kg body weight to C57BL/6 mice decreased the number of plaque-forming spleen cells in response to an injection of the thymus-dependent antigen sRBC. The response was dose dependent and lasted for at least 42 days. Luster et al. (1985) found a decrease in anti-sRBC plaque-forming spleen cell production in B6C3F1 and DBA/2 mice 5 days after single oral doses of 2 and 10 µg TCDD/kg body weight, respectively. A dose of 30 µg TCDD/kg body weight was needed to produce a significant antibody response to the thymus-independent antigen type III pneumococcal polysaccharide (sIII) in C57Bl/6 mice (Vecchi et al., 1980). With sRBC and the thymus-independent antigen trinitrophenylated Brucella abortus, Clark et al. (1981) found a depressed number of spleen plaque-forming cells in C57BL/6 mice only with a total dose of 40 µg/kg body weight given as four equal weekly doses. Chastain & Pazdernik (1985) demonstrated decreased numbers of plaque-forming spleen and bone marrow cells in response to the thymus-independent antigen trinitrophenylated lipopolysaccharide (LPS). Spleen and bone marrow

cells were collected from male C57BL/6 mice 7 days after a single ip dose of 30, 60, 90, or 120 µg TCDD/kg body weight. The decrease occurred at lower doses in the spleen cell assay but was more pronounced at higher doses in the bone marrow cell assay. The number of antibody-producing cells, measured asplaque-forming cells, following immunization with either sRBC or LPS was reduced in B6C3F1 mice treated with 1 and 5 µg TCDD/kg body weight (Tucker et al., 1986).

Table 51. Effects of TCDD on humoral-mediated immune responses in adult animals

| Species/strain Parameter measured[e] (reference) | Sex/age/weight[f] | TCDD exposure Frequency/route/dose | |
| --- | --- | --- | --- |
| **Mice** | | | |
| Swiss-Webster primary and secondary sRBC[a] antibody (Hinsdill et al., primary and secondary serum tetanus 1980) antitoxin level | F/4-7 weeks/NR | fed 10, 20, 50, 100, or 500 µg/kg continuously in the diet for 5 weeks | level, |
| C57BL/6J sRBC[a] plaque-forming spleen cells SIII[b] plaque-forming spleen cells (Vecchi et al., 1980) | M/6-8 weeks/NR | single ip dose of 1.2, 6, or 30 µg/kg body weight | anti- anti- |
| C57BL/6J sRBC[a] plaque-forming spleen cells TNP-BA[c] plaque-forming spleen cells (Clark et al., 1981) | M/6-8 weeks/NR | four weekly ip doses of 0.1, 1, or 10 µg/kg body weight | anti- anti- |
| C57BL/6 | M/8-10 weeks/NR | single ip dose of | anti- |

```
sRBC[a] plaque-forming spleen cells
     C3H/HeN                                  1.2, 6, or 30 µg/kg body
     DBA/2                                    weight
     AKR
     B6D2F1

  (Vecchi et al.,
    1983)

     C57BL/6          M/6-8 weeks/NR          single ip dose of 30, 60,      anti-
TNP-LPS[d] plaque-forming spleen cells

                                              90, or 120 µg/kg body weight   anti-

TNP-LPS[d] plaque-forming bone marrow
  (Chastain &                                                                cells
    Pazdernik, 1985)
```

Table 51. (contd - 2)

Species/strain	Sex/age/weight[f]	TCDD exposure	Parameter measured[e]
(reference)		Frequency/route/dose	

```
Mice (contd).
     DBA/2N           F/6-8 weeks/            single oral doses of 5,        anti-
sRBC[a] plaque-forming spleen cells
                      18-21 g                 10, or 50 µg/kg body weight
  (Luster et al.,
    1985)

     B6C3F1           F/6-8 weeks/            single oral doses of 0.2,      anti-
sRBC[a] plaque-forming spleen cells
                      18-21 g                 1, 2, 5, or 10 µg/kg body
  (Luster et al.,                             weight
    1985)

  Guinea-pigs
     Hartley          F/NR/256 g              eight weekly oral doses of
primary serum tetanus antitoxin level
                                              0.008, 0.04, 0.2, or

secondary serum tetanus antitoxin level
  (Vos et al., 1973)                          1.0 µg/kg body weight
```

<u>Rabbit</u>
       New Zealand   M/Adult/3 kg   eight weekly oral doses of
primary and secondary serum tetanus
                                            0, 0.01, 0.1, 1.0, or 10.0
antitoxin level
     (Sharma et al.,   µg/kg body weight
       1984)

---

   [a]   sRBC = sheep red blood cell.
   [b]   SIII = type III pneumoccal polysaccharide.
   [c]   TNP-BA = trinitrophenylated <u>Brucella</u> <u>abortus</u>.
   [d]   TNP-LPS = trinitrophenylated lipolysaccharide.
   [e]   = all parameters measured were decreased except for primary serum
tetanus antitoxin level in guinea-pigs
         (Vosal., 1973).
   [f]   M = male; F = female; NR = not reported.

    Table 52. Effects of TCDD on humoral-mediated immune responses in
maternally exposed animals

    Species/strain   Time of TCDD exposure   Route/
dose   Parameter measured-response
     (reference)

<u>Rats</u>
       Fisher-344 N   Prenatal day 18 and/or   oral/5 µg/kg body
weight   primary and secondary BGG[a]
                          postnatal days 0, 7, and
14   antibody level - no effect

     (Faith & Moore,
       1977)

       Fisher/Wistar   Prenatal day 18 and/or   oral/5 µg/kg body
weight   primary and secondary BGG[a]
                          postnatal days 0, 7, and
14   antibody level - no effect

     (Faith & Luster,
       1979)

<u>Mice</u>
       Swiss-Webster   four weeks before mating,   in the
diet/   primary and secondary sRBC[b]

                              throughout gestation and   1, 2.5, 5, 10, or 20 µ/
  kg   antibody level - no effect;

      (Thomas &
lactation   primary anti sRBC[b] plaque
        Hinsdill,
1979)   forming
spleen cells - decreased

_____

        a   = BGG - bovine gamma-globuline.
        b   = sRBC - sheep red blood cell.

        Only minor effects on antibody responses have been reported in
rodents maternally exposed to TCDD (Table 52). Single oral doses of 5
µg TCDD to pregnant Fisher-344 N (poor immunological responder) and
Fisher-Wistar rats (good immunological responder) on gestation day 18
and/or postnatal days 0, 7, and 14 did not affect the antibody reponse
to bovine gammaglobulin (BGG) in the offspring (Faith & Moore 1977;
Faith & Luster, 1979).

        Dietary exposure of female Swiss-Webster mice to 2.5 or 5 µg
TCDD/kg for 4 weeks before mating and throughout gestation and
lactation resulted in normal antibody production in the offspring but
in a decrease in anti-sRBC plaque-forming spleen cells.

        In vitro exposure of B6C3F1 spleen cells to 10^{-9} mol
TCDD/litre decreased the production of anti-sRBC plaqueforming cells
(Luster et al., 1984). The ED_{50} for this effect was found to be 7

nmol/litre when TCDD was present from the first day of culture (Tucker
et al., 1986). Similar activity to that of TCDD was found with
2,3,7-triCDD and 1,2,3,7,8-pentaCDD, whereas 2,8-diCDD and octaCDD
were without effect at concentrations up to 5×10^{-8} mol/litre
(Tucker et al., 1986).

        The doses producing 50% suppression of splenic IgM response to
sRBC in C57Bl/6 mice were 7.1 and 85 µg/kg body weight, respectively,
for 1,2,3,6,7,8-hexaCDD and 1,2,3,4,6,7,8-heptaCDD given as single
oral doses 2 days prior to sRBC challenge (Kerkvliet et al., 1985).
OctaCDD had no effect even at doses of 100 and 500 µg/kg body weight.

        Humoral immune responses of the rainbow trout (Salmo

gairdneri) were not significantly impaired even at doses of TCDD
that caused clinical toxicity (Spitsbergen et al., 1986).

7.4.5.3 Cell-mediated immunity

     Cell-mediated immunity (CMI) operates through specifically
sensitized lymphocytes and is transferred by these cells. Processes
included in this system are classical cell-mediated protective
immunity (which protects against fungi, bacteria, and viruses),
delayed type hypersensitivity, rejection of tumors and foreign tissues
such as transplants, and graft versus host response. Many assays, both
in vivo and in vitro, have been developed to test CMI functions.
Besides a reduction in the number of immunologically competent cells
after TCDD exposure (Gupta et al., 1973; Vos et al., 1973; Zinkl et
al., 1973) TCDD has been demonstrated to induce a decreased CMI
response in adults (Table 53) and even more in maternally exposed
animals (Table 54). Delayed hypersensitivity response, correlatingwith
decreased host resistance to infectious agents in man, was depressed
in rodents exposed to low levels of TCDD (Vos et al., 1973; Faith &
Moore, 1977; Sharma et al., 1978; Faith & Luster, 1979; Thomas &

Hinsdill, 1979; Hinsdill et al., 1980; Clark et al., 1981).
TCDD-exposure also adversely affects host susceptibility to bacteria,
viruses, tumor cells and endotoxins (Thigpen et al., 1975; Thomas &
Hinsdill, 1979; Hinsdill et al., 1980; Luster et al., 1980; Clark et
al., 1983).

     Depressed graft versus host response was reported in 2-month-old
C57BL/6 mice exposed to 4 weekly oral doses of 5 µg TCDD/kg body
weight (Vos et al., 1973), whereas no effect was seen in a subsequent
study on 1- and 4-months old C57BL/6Sch mice (Vos & Moore, 1974). In
the same study, Fisher-344 rats maternally exposed to TCDD showed
decreased graft versus host response and prolonged allograft-rejection
time. The latter effect was also demonstrated in maternally
TCDD-exposed C57BL/6Sch mice (Vos et al., 1973). Proliferative
responses of spleen and/or thymus lymphocytes, stimulated by mitogens
specific for the generation of B-lymphocytes and/or T-lymphocytes from
TCDD-exposed animals, were depressed both in adults (Vos & Moore,
1974; Sharma et al., 1978) and maternally exposed rodents (Vos &
Moore, 1974; Faith & Moore, 1977; Vos et al., 1978; Faith & Luster,
1979; Luster et al., 1980). However, Thomas & Hinsdill (1979) found no
effect on the lymphoproliferative response in offspring from
Swiss-Webster mice fed with up to 5 µg TCDD/kg diet 4 weeks before
mating and throughout gestation and lactation. Depressed
lymphoproliferative response is regarded as an extremly sensitive
indicator of immunotoxicity, rather than as a predictor of immune

dysfunction. Cytotoxic T-cell generation in response to allogeneic antigens has been demonstrated in male DBA/2, C57Bl/6, and B6D2F1 mice given four weekly ip injections of 1 ng/kg body weight (Clark et al., 1981, 1983). At this dose no effects were seen on delayed hypersensitivity, antibody response, thymus cellularity, or enzyme induction. The adverse effect of TCDD on CMI function seems to be an age-related phenomenon in rodents. In order to obtain a complete and persistent immune suppression, TCDD exposure must occur during ontogenesis of the immune system. In the initial experiments on the developing immune system, Vos & Moore (1974) exposed Fisher-344 rats to 1 mg TCDD/kg body weight on gestation days 11 and 18 and on postnatal days 4, 11, and 18, or to 5 µg TCDD/kg body weight on postnatal days 0, 7, and 14. CMI functions adversely affected included in vitro immune competence of spleen and thymus lymphoid cells, delayed hypersensitivity reaction, prolonged allograft-rejection times and reduced graft versus host activity. The immune suppression demonstrated persisted throughout the study, i.e., for 145 days. The depression of T-cell-dependent immune functions appeared to occur without helper-cell function being affected (Faith & Moore 1977).

Attempts to study direct effects of TCDD on lymphocytes in vitro were previously hampered by the low solubility of TCDD in physiological buffers (Matsumura & Benezet, 1973). Vos & Moore (1974)obtained no lymphoproliferative response in unstimulated or PHA- or concanavalin-A-stimulated rat thymus organ cells and mouse spleen cells when cultured in the presence of up to 20 ng TCDD/ml. Dencker et

al. (1985) demonstrated that mouse fetal thymus cells cultured in vitro in the presence of TCDD gave a similar response similarly to that occurring in vivo, i.e., with a dose-dependent inhibition of the time-dependent increase in the number of lymphoid cells ($EC_{50}=10^{-10}$ mol TCDD/litre). It could not be determined with certainty whether the decreased cell number caused by TCDD was due to reduced cell proliferation or to increased cell death. The TCDD-induced suppression of mitogen-stimulated lymphoproliferation has recently been demonstrated to be mediated by thymus epithelial cells (Greenlee et al., 1985).

7.4.5.4 Macrophage function

The primary pathway of endotoxin detoxification is thought to be macrophage-dependent. Thus the increased sensitivity to endotoxin following TCDD treatment (Vos et al., 1978; Thomas & Hinsdill, 1979) was suggestive of macrophage dysfunction. However, the number of peritoneal macrophages, as well as their capacity to mediate cytolytic and cytostatic effects, was not adversely affected by single ip doses

of 1.2, 6 or 30 µg TCDD/kg body weight to male C57Bl/6J mice
(Mantovani et al., 1980), nor was the ability of macrophages to reduce
nitroblue tetrazolium affected by four to five weekly oral doses of 50
µg TCDD/kg body weight in Swiss-Webster mice (Vos et al., 1978a).
Macrophage function does not appear to be altered by TCDD.

7.4.6 Myelotoxicity

TCDD treatment inhibits the bone marrow haematopoiesis in mice,
both in vivo and in vitro, by directly altering colony growth of
stem cells (Luster et al., 1980, 1985; Chastain & Pazdernik, 1985).
The bone marrow granulocyte-macrophage progenitor cell (CFU-GM)
production was reduced in B6C3F1 mice, receiving 1 µg TCDD/kg body
weight, but was unaffected in DBA/2 mice, even at a dose of 50 µg
TCDD/kg body weight (Luster et al., 1985). Chastain & Pazdernik (1985)
used the B-lymphocyte colony-forming unit assay to demonstrate
reductions in spleen and bone marrow B-cell function of C57BL/6 mice
exposed to single ip doses of TCDD in the range 30 to 120 µg TCDD/kg
body weight. Bone marrow B-cells tended to be more suseptible to TCDD
than spleen B-cells. A maternal oral dose of 5 µg TCDD/kg body weight
on the 14th day of gestation and postnatal days 1, 7, and 14 resulted
in a weak normocytic anaemia indicative of depressed erythrogenesis,
decreased bone marrow cellularity, and decreased stem cell
proliferation in B6C3F1 offspring, assessed 7 days after weaning
(Luster et al., 1980). In vitro exposure of B6C3F1 bone marrow cells
to 10^{-9} mol TCDD/litre resulted in decreased CFU-GM development and
decreased number of erythrocyte colony-forming units. The decrease in
CFU-GM occurred 1 day post-treatment and remained below control values
until 10 days post-treatment (Luster et al., 1985).

Table 53. Effects of TCDD on cell-mediated immunity responses in adult animals

Species/strain exposure (reference)	Sex/age/weight[d]	TCDD Parameter measured-response[d] Frequency/route/dose

Rats

CD	F/NR/185 g	six weekly oral doses of 0.2, 1.0, or 5.0 µg/kg

delayed hypersensitivity to tuberculin - NE

```
     (Vos et al., 1973)                              body weight


     Mice
        C57BL/6            M/6-8 weeks/NR           four weekly ip doses of
 delayed hypersensitivity to sRBC[a] - Dec;
                                                  0.1, 1.0, or 10.0 µg/kg

 delayed hypersensitivity to oxazalone - Dec;
     (Clark et al.,                               body
 weight                generation of alloantigen-specific cytotoxic
         1981)                                                        T-
 cells - Dec


         C57BL/6            M/NR/NR                 four weekly ip doses
 of      resistance to Herpes virus challenge - Dec;
                                                  0.001, 0.01, 1.0,
 or          generation of alloantigen-specific cytotoxic
     (Clark et al.,                               10.0 µg/kg body weight       T-
 cells - Dec
         1983)


         DBA/2              M/NR/NR                 four weekly ip doses
 of      generation of alloantigen-specific cytotoxic
                                                  0.001, 0.01, 0.1, 1.0, or      T-
 cells - Dec
     (Clark et al.,                               10.0 µg/kg body weight
         1983)


         Swiss-Webster     F/4-7 weeks/NR          five weeks feeding of
 diets   resistance to Salmonella typhimurium challenge - Dec;
                                                  containing 10, 50,
 or          resistance to Listeria monocytogenes challenge - Dec;
     (Hinsdill et al.,                            100 µg/kg body weight
 contact sensitivity to 2,4-dinitro-1-fluoro-
         1980)
 benzene - Dec
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     Table 53 (contd - 2)
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     Species/strain        Sex/age/weight[d]        TCDD
 exposure                Parameter measured-response[d]
     (reference)                                   Frequency/route/dose
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```
     Mice (continued)
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      C57BL/6J          M/6-8 weeks/NR          single ip doses of 1.2,
 number of peritoneal macrophages - Dec;
                                               6 or 30 µg/kg body weight
 number of splenic natural killer cells - Dec;
     (Mantovani
 macrophage mediated cytolysis - NE;
       et al.,
 1980)                                                        macrophage
 mediated cytostasis - NE


      C57BL/6          M/NR/NR                 four weekly ip doses
 of      generation of allospecific cytotoxic T-cells - Dec
      DBA                                      0.001 µg/kg body weight       - NE
      B6D2F₁                                                                 - Dec


     (Nagarkatti
       et al., 1984)


      CD-1             M/NR/28                 two, four, or eight
 weekly    lymphoproliferative response of PHAᵇ- and PWMᶜ-
                                               doses of 0.01, 0.1, 1.0,
 or   stimulated splenic cells - Dec
      (Sharma &                                10.0 µg/kg body weight
       Gehring, 1979)


      C57BL/6Jfh       M/4 weeks/NR            four weekly oral doses
 of      resistance to Salmonella bern challenge - Dec;
                                               0.5, 1.0, 5.0, 10.0,
 or      resistance to Herpes virus challenge - NE
      (Thigpen et al.,                         20.0 µg/kg body weight
       1975)


      C57BL/6          M/2 months/             four weekly oral doses of
 graft versus host activity - Dec
                        24.4 g                 0.2, 1.0, 5.0, or 25.0
      (Vos et al.,                             µg/kg body weight
       1973)


      C57BL/6Sch       M/1 month/NR            four weekly oral doses
 of      lymphoproliferative response of PHAᵇ-stimulated
                                               1.0, 5.0, or 25.0 µg/kg
 spleen cells - Dec;
      (Vos & Moore,                            body weight
 graft versus host activity - NE
       1974)
```

Table 53 (contd - 3)

Species/strain   Sex/age/weightd   TCDD
exposure   Parameter measured-responsed
   (reference)   Frequency/route/dose

Mice (continued)
     C57BL/6Sch   M/4 months/NR   six weekly oral doses
of   lymphoproliferative response PHA-stimulated
                                          1.0, 5.0, or 25.0 µg/kg

spleen cells - NE
   (Vos & Moore,   body weight
graft versus host activity - NE
     1974

     Swiss   M/3-4 weeks/NR   four or five weekly
oral   resistance of Listeria monocytogenes - NE;
                                          doses of 50 µg/kg body

number of peritoneal macrophages - NE;
   (Vos et al.,
weight   macrophage reduction of nitroblue tetrazodium - NE
     1978a)

   Guinea-pigs
     Hartley   F/NR/256 g   eight weekly oral doses
delayed hypersensitivity to tuberculin - Dec
                                          of 0.008, 0.04, 0.2, or
   (Vos et al, 1973)   1.0 µg/kg body weight

   Rabbits
     New Zealand   M/adult/3 kg   eight weekly oral doses
delayed hypersensitivity to tuberculin - Dec
                                          of 0.01, 0.1, 1.0, or
   (Sharma et al.,   10.0 µg/kg body weight
     1984)

a   sRBC = sheep red blood cells.
b   PHA = phytohaemagglutinin.
c   PWM = poke weed.
d   NR = not reported; NE = no effect; Dec = decreased; M = male; F
= female.

Table 54. Effects of TCDD on cell-mediated immunity responses in
maternally exposed animals

Species/strain (reference)	TCDD exposure Frequency/route/dose	Parameter measured - response[f]	Age when tested[g]
Rats			
Fisher/Wistar (Faith & Luster, 1979)	5 µg/kg body weight on gestation day 18 and on postnatal days, 0, 7, and 14, 5 µg/kg body weight on postnatal days 0, 7, and 14	lymphoproliferative response of PHA[a]- and ConA[b]-stimulated spleen and thymus cells - Dec; delayed hypersensitivity to tuberculin - Dec	25 days or 14 days
Fisher-344 (Faith & Moore, 1977)	5 µg/kg body weight on gestation day 18 and on postnatal days 0, 7, and 14, 5 µg/kg body weight postnatal days 0, 7, and 14	lymphoproliferative response of PHA[a]- and Con[b]-stimulated spleen and thymus cells - Dec; delayed hypersensitivity to oxazolone - Dec	25 days or 14 days
Fisher 344 (Vos & Moore, 1974)	1 µg/kg body weight on gestation days 11 and 18 and on postnatal days 4, 11 and 18 5 µg/kg body weight on postnatal days 0, 7 and 14	lymphoproliferative response of PHA[a]-stimulated spleen cells and of PHA[a]- and ConA[b]-stimulated thymus- cells - Dec	25 days or 14
Mice			
B6C3F$_1$ [c]	1, 5, or 15 µg/kg body weight on		

```
NR              lymphoproliferative response of
                              gestation day 14 and on postnatal days
1,                    mitogen-stimulated spleen cells:
    (Luster et al.,      7, and
14                                                    PHA[a] - Dec;
      1980)
ConA[b] - Dec;
                                                                        LPS[d]

- NE;
                                                                        Macrophage

proliferation - NE;                                                     Phagocytizing

ability - NE;                                                           Resistance

to Listeria monocyto-
                                                                        genes
- Dec;
                                                                        Resistance
to PYB6 tumor cells - Dec
```

_____

    Table 54 (contd).

_____

```
    Species/strain       TCDD exposure                            Age
when      Parameter measured - response[f]
    (reference)          Frequency/route/dose                     tested[g]
```

_____

```
    Mice (continued)
      Swiss-Webster    Feeding 1, 2.5, or 5 µg TCDD/kg            5-6
weeks    Contact sensitivity to 2,4-dinitro-
                              4 weeks before mating,
throughout                         1-fluorobenzene - Dec;
    (Thomas &         gestation, and 3 weeks
postnatally                          lymphoproliferative response of
      Hinsdill,
1979)                                                 PHA[a]- and
ConA[b]-stimulated spleen
                                                                        and
thymus cells - NE;
                                                                        Resistance
to Salmonella typhimurium
                                                                        endotoxin
- Dec; Resistance
                                                                        to
```

<u>Listeria</u> <u>monocytogenes</u> - NE

     C57BL/6Sch   2 or 5 µg/kg body weight on gestation days   23
days   Skin graft assay - prolonged skin
                    14 and 17 and on postnatal days 1, 8, and
15   graft rejection time
    (Vos et al., 1974)

     Swiss   10 µg/kg body weight on postnatal days   22
days   lymphoproliferative response of
                    1, 4, 8, 11, 15, and
18   PHA[a]-, ConA[b]- &
    (Vos et
al.,
PWM[e]-stimulated thymus cells - Dec
   1978a)

---

[a]   PHA = phytohaemagglutinin.
[b]   ConA = concanavalin A.
[c] $B6C3F_1$ = progeny to female C57BL/6N and male C3H mice.
[d]   LPS = lipopolysaccharide.
[e]   PWM = poke weed.
[f]   Dec = decreased, NE = no effect.
[g]   NR = not recorded.

7.4.7 Effects on the intermediary metabolism

    Changes in intermediary metabolism have been demonstrated in
TCDD-treated experimental animals. The circulating concentration of
glucose in TCDD-treated rats was decreased relative to <u>ad</u>
<u>libitum</u>-fed (Zinkl et al., 1973; Schiller et al., 1985) and pair-fed
(Gasiewicz et al., 1980; Potter et al., 1983) control rats.
TCDD-treated and pair-fed controls (both groups were schedule fed) had
similar concentrations of serum glucose (Christian et al., 1986b). The
TCDD-induced hypoglycaemia was not caused by altered pancreatic
function, as judged by insulin and glucagon levels (Potter et al.,
1983).

    Reduced hepatic glycogen content, compared to the value in
control rats, was reported in Sprague Dawley rats 16 days after a
single ip dose of 20 µg TCDD/kg body weight (Weber et al., 1983).

However, in studies by Christian et al. (1986b), compared to pair-and schedule-fed control rats, TCDD-treated (75 µg/kg body weight daily) Sprague Dawley rats had significantly increased hepatic glycogen levels but unaffected cardiac and muscle (gastrocnemius) levels of glycogen 2-8 days post-treatment.

TCDD-treated rats maintained a normal overall nitrogen balance, as judged by urinary urea, creatinine, and ammonia levels, but exhibited changes in certain plasma protein levels (Christian et al., 1986b).

Elevated circulating cholesterol levels were found in TCDD-treated rats (Albro et al., 1978; Poli et al., 1980; Schiller et al., 1985), whereas circulating free fatty acids and triacylglycerols were decreased in TCDD-treated rats, when compared to pair-and schedule-fed control rats (Christian et al., 1986b).

A marked accumulation of hepatic lipid has been found in rats after single doses of TCDD (Cunningham & Williams, 1972; Gupta et al., 1973; Albro et al., 1978; Schiller et al., 1985). At a sublethal dose of TCDD, hepatic triglyceride and free fatty acid levels were elevated already one day after dosing. Abnormal lipid deposition patterns persisted for at least 2 months (Albro et al., 1978). The increased level of triglycerides in the liver (Schiller et al., 1985; Christian et al., 1986b) of TCDD-treated rats was not accompanied by increases in cardiac or muscle (gastrocnemius) triacylglycerol levels (Christian et al., 1986b). Hepatic lipid synthesis in Wistar rats, measured as the 1-h incorporation of ^{3}H-acetate, was not affected by TCDD treatment when studied 7 days after exposure to 10 µg TCDD/kg body weight (Cunningham & Williams, 1972).

Mice (both C57BL/6 and DBA/2 strains) responded to TCDD treatment with dose-dependent decreases in serum levels of glucose, cholesterol, and triglycerides, and increases in hepatic triglycerides, whereas serum glycerol and free fatty acid levels were unaffected (Chapman & Schiller, 1985).

Hartley guinea-pigs given a lethal ip dose of 2 µg TCDD/kg body weight had significantly increased circulating levels of cholesterol esters, triglycerides, and phospholipids but a normal free fatty acid level 7 days post-treatment. The increase in serum lipids was accompanied by a pronounced increase in low density lipoproteins, particularly the very low density lipoprotein fraction (Swift et al., 1981). The plasma cholesterol and triglyceride levels were elevated also when TCDD-treated guinea-pigs were compared to pair-fed controls (Gasiewicz & Neal, 1979).

A TCDD-induced increase in plasma triglyceride levels was also noted in New Zealand rabbits, fed 20 µg TCDD/kg body weight, whereas plasma cholesterol was unaffected 12 weeks after dosing (Lovati et al., 1984). TCDD treatment did not alter the liver lipid levels (free and esterified cholesterol, triglycerides, and phospholipids), but the triglyceride level was significantly increased. These results were valid both for rabbits fed normal chow and those fed a cholesterol-rich (0.5%) diet. Golden Syrian hamsters exposed to 1000 µg TCDD/kg body weight, orally or ip, had elevated plasma cholesterol levels until 20 days after exposure but normal levels on day 50, whereas serum triglyceride levels were normal until day 20 and then became significantly lower than those of controls (Olson et al., 1980b).

7.4.8 Enzyme induction

Primarily, TCDD has been found to increase enzyme activities although observations on enzyme inhibition have also been made. Since the first reports of enzyme systems as targets for TCDD (Buu-Hoi et al., 1971a, 1972b; Greig, 1972; Poland & Glover, 1973b,c), enzyme induction has become the most extensively studied biochemical response produced by TCDD. The mixed function oxidase system (MFO), capable of metabolizing both endogenous and foreign lipophilic compounds to more polar products, has been the most thoroughly investigated one, and arylhydrocarbon hydroxylase (AHH) and 7-ethoxy-resorufin 0-deethylase (EROD) are the most frequently assayed enzyme activities in this system. TCDD has also been reported to affect UDP-glucuronosyl transferases (UDPGT) (Thunberg et al., 1980, 1984) and glutathione-S-transferases (GT) (Manis & Apap, 1979), which are multifunctional enzyme systems involved in conjugating a wide variety of compounds.

Most studies have been performed with microsomal enzymes, but TCDD has also been found to have effects on enzymes in the cytosolic fraction. It seems that TCDD produces organ-specific effects, and although, quantitatively, hepatic enzyme induction is of more concern than extrahepatic enzyme effects, the latter may qualitatively be as important. Studies in different species have revealed that enzyme induction due to TCDD exposure also is a species-specific phenomenon.

Time course studies have shown that maximal increases in enzyme activities are reached within 3 to 4 days post-treatment. After a lag period of about 2 to 3 weeks, enzyme activities begin to return to normal levels (Hook et al., 1975a,b; Lee & Suzuki, 1980; Lucier et al., 1973; Poland & Glover, 1973a).

According to Kitchin & Woods (1979), TCDD-induced AHH activity did not reach the normal level until 6 months after rats were exposed to a single daily dose of 2 µg/kg body weight.

Hook et al. (1975a) found no apparent dependence on age when studying AHH induction in CD rats which were 10 to 335 days old at the time of exposure to 25 µg TCDD/kg body weight.

Several investigators have studied the relative potency of various PCDDs and PCDFs to induce AHH and/or EROD activities (Bradlaw et al., 1980; Poland et al., 1976; Bandiera et al., 1984a,b; Sawyer & Safe, 1985; Mason et al., 1986). They found an apparent structure-activity relationship between the location of the halogen atoms on the dibenzo-p-dioxin molecule and the ability to induce AHH activity both in vivo and in vitro. Isomers with halogens at the four lateral ring positions produced a greater biological response than those with halogens at three lateral ring positions, while two lateral halogen atoms seemed to be insufficient to produce a biological response. TCDD was the most potent enzyme inducer of the compounds tested.

On a molecular basis TCDD is the most potent MFO-inducing compound known and MFO induction seems to be the most sensitive biochemical response produced by this chemical. According to Kitchin & Woods (1979), induction in the rat takes place after a single daily dose of only 0.002 µg TCDD/kg body weight. In the guinea-pig (the animal most sensitive to TCDD toxicity), MFO induction has been observed, but the induced activities were low even at lethal doses (Hook et al., 1975a). Neither is there a correlation in cell cultures between induction of MFO and toxicity. Furthermore, it is known that metabolites of TCDD are less toxic and more readily excreted than the parent compound (see section 8.1.5). Thus, TCDD-induced MFO activities represent a detoxification process rather than one leading to toxic effects.

However, induction of MFO activities might potentiate the toxicity of other foreign compounds requiring metabolic transformation by the MFO system before they can exert their toxic effect. A number of studies have shown that induction of MFO activities alters the

metabolism of the model xenobiotic, benzo(a)pyrene by increasing the rate of microsomal metabolism, changing the metabolic profile to more toxic metabolites, and increasing the extent of covalent binding to liver microsomes (Berry et al., 1976, 1977; Uotila et al., 1978). TCDD, applied topically or subcutaneously increased the

carcinogenicity of 3-methyl cholanthrene (MC) in DBA/2 mice (Kuori, 1978), but decreased the carcinogenicity of 7,12-dimethylbenz(a)anthracene (DMBA) in CD-1 mice (DiGiovanni et al., 1979a). These authors suggested that TCDD induces the MFO system and thus increases activation of MC to the ultimate carcinogen, as well as inactivation of DMBA, which would explain these effects.

Furthermore, increased MFO activities might adversely affect important metabolic pathways of endogenous compounds. The effects of TCDD on enzyme activities, both MFO and others, involved in such biological pathways as keratinization, steroid metabolism, lipid metabolism, plasma membrane function and porphyrin metabolism, are discussed under separate sections. The minute quantities of TCDD required for maximal enzyme induction or suppression, the long duration of the effect, and the stereospecific requirements suggest a specific interaction of TCDD with a cellular species, possibly at the gene level. Accordingly considerable research has been directed toward the study of the genetic regulation of AHH induction by TCDD. A hepatic cytosolic species that bound TCDD has been suggested as the receptor for the hepatic AHH activity. Numerous studies of this cytosolic receptor in several species and tissues have been performed and it seems that there is a structural gene, the Ah locus, for this receptor, which is responsible for the expression of various enzyme activities (see sections 7.8 and 7.8.1).

7.4.8.1 Studies on rats

The effect of TCDD on enzyme activities has been most extensively investigated in the rat. In the liver, TCDD has been shown to increase both the content of cytochrome P-450 (Lucier et al., 1973, 1986; Poland & Glover, 1974a,b; Hook et al., 1975a; Aitio & Parkki, 1978; Kitchin & Woods, 1979; Madhukar & Matsumura, 1981; Goldstein & Linko, 1984) and cytochrome b5 (Lucier et al., 1973; Hook et al., 1975a), as well as the microsomal enzyme activities involved in the oxidative transformation and conjugation of xenobiotics, e.g., aniline hydroxylase, arylhydrocarbon hydroxylase (AHH), biphenyl hydroxylase, 7-ethoxycoumarin-0-deethylase (ECOD), EROD, and UDPGT. These enzyme activities have been investigated in a vast number of studies, some of them quoted in Table 55. Goldstein & Linko (1984) demonstrated that TCDD induced two isozymes of cytochrome P-450 (P-448) in the liver but only one of these in extrahepatic tissues of young Sprague Dawley rats 2 days after a single oral dose of 25 µg/kg body weight.

Table 55. Studies demonstrating in vivo induction of mixed function oxidases and UDP-glucuronosyltransferases in TCDD-exposed strains of rats

Enzyme activity	Rat strain	Reference
Aniline hydroxylase	SD	Beatty et al. (1978)
	CD	Lucier et al. (1973); Hook et al. (1975a)
Aryl hydrocarbon hydroxylase	SD	Poland & Glover (1973a, 1974a); Beatty et al. (1978); Manis & Apap (1979); Haaparanta et al. (1983); Thunberg et al. (1984); Lucier et al. (1986); Ahlborg et al. (1987)
	CD	Lucier et al. (1973); Hook et al. (1975a); Kitchin & Woods (1979)
	Wistar	Nagayama et al. (1983); Keys et al. (1985); Bannister et al. (1986); Farrell & Safe (1986); Mason et al. (1986); Tsyrlov et al. (1986)
Biphenyl hydroxylase	CD	Hook et al. (1975a,b,); Kitchin & Woods (1979)
7-Ethoxycoumarin-O-deethylase	Wistar	Aitio & Parkki (1978)
7-Ethoxyresurofin-O-deethylase	SD	Haaparanta et al. (1983)
	CD	Kitchin & Woods (1979)
	Wistar	Keys et al. (1985); Bannister et al. (1986); Farrell & Safe (1986); Mason et al. (1986)
UDP-glucuronosyl-transferase: p-nitrophenol	SD	Thunberg et al. (1980, 1984); Ahlborg et al. (1987)
	CD	Lucier et al. (1973, 1975a, 1986); Hook et al. (1975a)

Table 55 (Cont.) Studies demonstrating in vivo induction of mixed

function oxidases and UDP-glucuronosyltransferases in TCDD-exposed
strains of rats

Enzyme activity	Rat strain	Reference
UDP-glucuronosyl-transferase:		
p-nitrophenol (cont'd.)	Wistar	Aitio et al. (1979); Thunberg & Híkansson (1983)
o-aminophenol	Wistar	Aitio et al. (1979)
4-methylumbelli-ferone	Wistar	Aitio & Parkki (1978)

Microsomal glutathion-s-transferase (GT) did not respond to
TCDD (Aitio & Parkki, 1978; Mukitari et al., 1981; Baars et al.,
1982), but cytosolic GT was induced both by a single dose of 17 µg
TCDD/kg body weight 2 days post-treatment (Manis & Apap, 1979), and by
near lethal or lethal doses 1 and 6 days after dosing (Mukitari et
al., 1981; Baars et al., 1982; Hassan et al., 1983). Glutathione
reductase was also increased, while glutathione peroxidase, both total
and Se-dependent, and the content of reduced glutathione were reduced
by TCDD treatment (Hassan et al., 1983, 1985a,b,c).

The following hepatic enzyme activities involved in drug
metabolism have been reported to be unaffected by TCDD treatment in
the rat: N-and O-demethylation (Lucier et al., 1973; Poland &
Glover, 1973a; Hook et al., 1975a; Beatty et al., 1978; Kitchin &
Woods, 1979; Madhukar & Matsumura, 1981), epoxide hydratase (EH)
(Aitio & Parkki, 1978), ß-glucuronidase (Lucier et al., 1973, 1975)
and NADPH cytochrome c reductase (Poland & Glover, 1974; Aitio &
Parkki, 1978; Kitchin & Woods, 1979; Madhukar & Matsumura, 1981). The
glucuronide conjugation of bilirubin (Aitio et al., 1979), estrone,
and testosterone (Lucier et al., 1975a) by liver microsomes from
TCDD-treated rats was not different when compared to control rats.

Some hepatic enzyme activities not belonging to the MFO system,
which are affected by TCDD treatment include aldehyde dehydrogenase
(Deitrich et al., 1978; Lindahl et al., 1978), delta-aminolevulinic
acid synthetase (see section 7.4.3), DT-diaphorase (Beatty & Neal,
1977), transglutaminase (see section 7.4.4), ornithine decarboxylase
(Nebert et al., 1980; Potter et al., 1982; Farrell & Safe, 1986),

plasma membrane ATPases (see section 7.4.2.2), porphyrinogen

carboxylase (see section 8.4.3), prostaglandin synthetase (see section
7.4.9), enzymes involved in testosterone metabolism (see section
7.4.9), and RNA polymerase (Kurl et al., 1982).

Prenatal and postnatal exposure via milk to TCDD, at doses of 3
µg/kg body weight to pregnant rats on days 5, 10 and 16 of gestation,
induced hepatic AHH and UDPGT activities in the offspring. The effect
was seen 8 days post-partum and persisted for at least 2 weeks. The
inductive effect was due both to exposure to TCDD via milk and to the
activation of an inducing mechanism after birth. Fetal liver AHH was
slightly increased during late gestation, although the UDPGT activity
and the cytochrome P-450 content were not (Lucier et al., 1975b).

Administration of 2.5 µg TCDD/kg body weight to pregnant rats on
day 17 of gestation increased the AHH- and N-hydroxylation activities
and cytochrome P-450 content in the fetal liver on day 20 of gestation
(Berry et al., 1976).

AHH induction due to TCDD has been reported to occur also in the
brain (Hook et al., 1975a), kidney (Poland & Glover, 1973a; Hook et
al., 1975a; Aitio & Parkki, 1978; Potter et al., 1982; Nagayama et
al., 1983), lung (Poland & Glover, 1973a; Hook et al., 1975a; Aitio &
Parkki, 1978; Nagayama et al., 1983), prostate (Lee & Suzuki, 1980;
Haaparanta et al., 1983; Nagayama et al., 1983), thymus (Nagayama et
al., 1983), and intestine (Poland & Glover, 1973a; Hook et al.,
1975a), but intestinal AHH activity was found to be unaffected by 17
and 20 µg TCDD/kg body weight (Aitio & Parkki, 1978; Manis & Apap,
1979). Testicular (Poland & Glover, 1973a; Hook et al., 1975a; Aitio
& Parkki, 1978) and adrenal (Guenthner et al., 1979) AHH activities
were not induced by sublethal doses of TCDD. The O-deethylation
activity in kidney, lung, and prostate was increased, but no effect
was seen on the activity in testes or intestine (Aitio & Parkki, 1978;
Haaparanta et al., 1983). UDPGT activities in kidney, lung, intestine,
and brain were increased, while no effect was seen on testicular UDPGT
(Hook et al., 1975a). Similar results were reported by Aitio & Parkki
(1978), though in their study intestinal UDPGT was not affected.

Renal biphenyl hydroxylation activity has been found to increase
after TCDD treatment, but no effect on this enzyme activity was seen
in lung, intestine, brain, or testes (Hook et al., 1975a). Elevated
levels of cytochrome P-450 were found in prostate (Lee & Suzuki, 1980)
and mammary gland (Rikans et al., 1979), but not in adrenals
(Guenthner et al., 1979). Less testicular cytochrome P-450 was found
after a single dose of 25 µg TCDD/kg body weight (Tofilon & Piper,

1982). The GSH tranferase activity was increased in the lung but not in kidney, intestine, testes (Aitio & Parkki, 1978), or prostate (Lee & Suzuki, 1980).

Neither EH nor NADPH cytochrome c reductase were inducible by TCDD in kidney, lung, intestine, testes (Aitio & Parkki, 1978), mammary gland (Rikans et al, 1979), or prostate (Lee & Suzuki, 1980). The ED_{50} values for hepatic AHH and EROD induction were determined in immature male Wistar rats 13 days after a single ip dose of 2,3,7-triCDD, TCDD, 1,3,7,8-tetraCDD, 1,2,3,7,8-pentaCDD, 1,2,4,7,8-pentaCDD or 1,2,3,4,7,8-hexaCDD (Mason et al., 1986). The order of enzyme-inducing capacity was TCDD > 1,2,3,7,8-pentaCDD > 1,2,3,4,7,8-hexaCDD > 1,2,4,7,8-pentaCDD > 2,3,7-triCDD > 1,3,7,8-tetraCDD (Table 56).

7.4.8.2 Studies on mice

Enzyme induction studies in mice have been performed mainly with two strains genetically separated at the Ah locus, thus making them responsive (C57Bl/6 (B6)) or non-responsive (DBA/2 (D2)) to induction of hepatic cytochrome P-450-related enzyme activities by aromatic hydrocarbons, e.g., 3-methyl-cholanthrene (3-MC). However, the extraordinary potency of TCDD for enzyme induction revealed increased hepatic cytochrome P-450 content as well as AHH and O-deethylase activities both in B6 and D2 mice after sublethal exposure to TCDD (Poland & Glover, 1974a,b,c; Jones & Sweeney, 1977; Greenlee & Poland, 1978). Studies of MFO induction in five responsive and five non-responsive strains of mice by Poland & Glover (1974a,b,c) revealed that there were no consistent differences between the strains when considering the extent to which TCDD induced AHH, O-deethylase, N-demethylase and O-demethylase activities in the liver, kidney, lung, skin, or bowel. The ED_{50} for hepatic AHH induction was determined to be 10^{-9} mol/kg body weight in the responsive strain and > 10-8 mol/kg body weight in the non-responsive strain (Poland & Glover, 1975). Fully induced hepatic AHH activity was obtained both in responsive (C57Bl/6 and AKR/Qdj) and non-responsive (DBA/2 and DDD) strains of mice 3 days after an ip dose of 30 µg TCDD/kg body weight (Nagayama et al., 1985a). Both AHH and EROD were induced in C57Bl/6 mice 7 days after an ip dose of 0.32 µg/kg body weight (Bannister et al., 1986). Both hepatic AHH and ornithine decarboxylase activities were similarly induced in C57Bl/6 and DBA/2 mice after a single ip dose of 100 µg TCDD/kg body weight, but at 2 µg TCDD/kg body weight these enzymes were induced only in the C57Bl/6 strain (Nebert et al., 1980). Two daily doses of 0.1 µg TCDD, topically applied, increased the epidermal AHH activity in two strains of hairless mice (Puhvel et

al., 1982). Contrary to observations in rats, TCDD induces testicular AHH activity both in B6 and D2 mice 40h after an ip dose of 50 µg/kg body weight (Mattison & Thorgeirsson, 1978).

Table 56. Structure activity relationships for some PCDDs

PCDD Congener	In vitro EC$_{50}$ values (M)[a]			In vivo ED$_{50}$ values (mmol/kg)[b]				LD$_{50}$[c] µg/kg body weight
	Receptor binding	AHH	EROD Guinea-pig	AHH weight	EROD atrophy	Body	Thymic	
1-	$> 1.0 \times 10^{-4}$	$> 1.0 \times 10^{-4}$	$> 1.0 \times 10^{-4}$					
2,8-	3.2×10^{-6}	$> 1.0 \times 10^{-4}$	$> 1.0 \times 10^{-4}$					$> 300\,000$
1,2,4-	1.3×10^{-5}	4.8×10^{-5}	2.2×10^{-6}					
2,3,6-	2.2×10^{-7}							
2,3,7-	7.1×10^{-8}	3.6×10^{-7}	1.4×10^{-7}	19.6	19.6		98.1	29 400
1,2,3,4-	1.3×10^{-6}	3.7×10^{-6}	2.4×10^{-6}					
1,2,3,8-		6.1×10^{-7}						
1,3,7,8-	7.9×10^{-7}	5.9×10^{-7}	3.2×10^{-7}	31.2	77.6	132	100	
2,3,6,7-	1.6×10^{-7}	6.1×10^{-8}	1.1×10^{-8}					
2,3,7,8-	1.0×10^{-8}	7.2×10^{-11}	1.9×10^{-10}	0.004	0.003	0.05	0.09	2
1,2,3,4,7-	6.4×10^{-6}	6.6×10^{-7}	8.2×10^{-7}					
1,2,3,7,8-	7.9×10^{-8}	1.1×10^{-8}	1.7×10^{-8}	0.031	0.056	0.62	0.17	3.1
1,2,4,7,8-	1.1×10^{-6}	2.1×10^{-8}	1.1×10^{-8}	2.82	0.56	34	11.2	1125
1,2,3,4,7,8-	2.8×10^{-7}	2.1×10^{-9}	4.1×10^{-9}	0.03	0.130	1.63	1.07	72.5

     1,2,3,6,7,8-c 5.7×10^{-10}
 3.1×10^{-8}
                                                                    70-100
     1,2,3,7,8,9-c 1.4×10^{-9}
 4.6×10^{-8}
                                                                    60-100
     1,2,3,4,6,7,8c
 1.3×10^{-7}
 > 600
     1,2,3,4,6,7,9-c 3.7×10^{-6}
     1,2,3,4,6,7,8,9- $> 1.0 \times 10^{-5}$ $> 1.0 \times 10^{-4}$ $> 1.0 \times 10^{-4}$

---

     [a]   Estimated concentrations needed to displace 50% of ^{3}H-TCDD bound to
liver cytosol receptor from Wistar
          rats and to produce 50% maximum enzyme induction in the rat hepatoma 11-
4-II E cell line (Bradlaw & Casterline,
          1979; Mason et al., 1986).
     [b]   Studies in immature male Wistar rats (Mason et al., 1986).
     [c]   McConnell et al., 1978b.

7.4.8.3 Studies on guinea-pigs

     The guinea-pig, the species most sensitive to the toxic effects
of TCDD, does not respond with liver toxicity or with extensive
enzyme induction.

     Hook et al. (1975a) investigated the effect of a single oral dose
of 0.175 µg TCDD/kg body weight on Hartley guinea-pig MFO and UDPGT
activities in liver, kidney, and lung. AHH induction was found only in
the kidney. In none of the tissues was there an effect on UDPGT
activity. The biphenyl 4-hydroxylase activity was increased in all
tissues, whereas hepatic biphenyl 2-hydroxylase was decreased. With
three daily doses of 1 µg TCDD/kg body weight, Hassan et al. (1983)
found an increase in hepatic AHH 6 days post-treatment. They also
found slightly increased in vitro lipid peroxidation but no effect
on glutathione content or on the enzyme activities facilitating
peroxidation, reduction, or transfer of glutathione. The DT-diaphorase
activity was not affected by a single oral dose of 0.6, 3.0, or 6.0 mg
TCDD/kg body weight (Beatty & Neal, 1977). The maximal increases were
4.4 and 22 times for AHH and EROD activities, respectively. The
testicular cytochrome P-450 content in Hartley guinea-pigs was

decreased by 50% one day after a single dose of 1 mg TCDD/kg body
weight. The effect persisted for at least 9 days (Tofilon, 1980). No
effect was seen on microsomal haeme content or on the activities of
NADPH-cytochrome C reductase and sorbitol dehydrogenase, the marker
enzyme for testicular protein synthesis. Thus, the decrease in
cytochrome P-450 induced by TCDD does not seem to be a nonspecific
inhibition of protein synthesis.

7.4.8.4 Studies on rabbits

     Studies on enzymes in rabbits have been performed in the New
Zealand albino strain exposed to single doses of 10 to 30 mg TCDD/kg
body weight for 1 to 5 days. Both in adults (Johnson &
Muller-Eberhard, 1977a,b) and in neonates exposed in utero (Norman
et al., 1978; Kohli & Goldstein, 1981), TCDD increased the content of
cytochrome P-450. It also induced the formation of immunologically
distinct cytochromes P-450 in adult and neonatal liver (Norman et al.,
1978). Increased cytochrome P-450 was observed in the kidney but not
in the lung (Liem et al., 1980; Kohli & Goldstein, 1981). Renal and
pulmonary cytochrome P-450 reductase, investigated by Liem et al.
(1980), were not affected by TCDD treatment. Data on MFO induction and
suppression are conflicting. Liem et al. (1980) reported increased AHH
and O-deethylase activities in lung and kidney, whereas Hook et al.
(1975a) saw no effect on the AHH activity in the lung and reported a
decrease in hepatic AHH activity after a single oral dose of 0.5 µg
TCDD/kg body weight. Biphenyl 4-hydroxylase induction was seen in the
liver by Johnson et al. (1979). Hook et al. (1975a) detected such
induction in lung, but no effect in the liver and kidney (Hook et al.

1975a). Furthermore, Hook et al. (1975a) reported no effects on
biphenyl-2-hydroxylation and UDPGT activities in liver, kidney, or
lung. A decrease in hepatic, but not in renal or pulmonary
N-demethylation, was found by these authors.

7.4.8.5 Studies on hamsters

     Golden Syrian hamsters are among the animals most resistant to
acute lethal effects induced by TCDD. Although the liver is a target
tissue, hepatic enzyme induction has barely been studied in this
species. When given an oral dose of 200 µg/TCDD/kg body weight for a
period of 3 days, increased hepatic glutathione-S-transferase and
glutathione reductase activities were found, but no effects were seen
on AHH or glutathione peroxidase activities. Neither the hepatic level
of glutathione nor the in vitro lipid peroxidation were affected
(Hassan et al., 1983). The ED_{50} values for induction of hepatic ECOD

and reduced NAD(P), menadione oxidoreductase activities, and

cytochrome P-450 content in male Golden Syrian hamsters were 1.0, 2.0, and 0.5 µg TCDD/kg body weight, i.e. extremely low doses as compared to doses that produce tissue damage and lethality in this species (Gasiewicz et al., 1986).

7.4.8.6 Studies on cows

Three dairy Holstein cows (500-600 kg) received a single oral dose of 0.05 (two cows) or 7.5 µg TCDD/kg body weight (Jones et al., 1986). The cow receiving the high dose was killed on day 7 and those receiving the low dose on day 14. AHH and EROD activities were markedly induced in the high-dose but not in the low-dose animals.

7.4.8.7 Studies on chick embryos

AHH and delta-aminolevulinic acid synthetase in the chick embryo have been reported to be extremely sensitive to the inductive effects of TCDD (Poland & Glover, 1973b,c). Maximal induction occurred with 155 pmol TCDD/egg. The induction was relatively long lasting, with 70% of the maximum induced activity present 5 days following a single dose of TCDD. Structure-activity studies demonstrated a good correspondence between the toxicity and induction potency of a series of dibenzo-p-dioxin congeners (Poland & Glover, 1973c).

ED^{50} values for the induction of hepatic microsomal EROD, AHH, and 4-dimethylaminoantipyrine-N-demethylase in 2-week-old white Leghorn cockerels on day 5 after TCDD exposure were 778, 302, and 561 ng/kg body weight, respectively, and aldrin epoxidase was inhibited by TCDD treatment (Sawyer et al., 1986). Hepatic and cardiac EROD activities were increased in white Leghorn chicken embryos exposed to TCDD in ovo at doses between 1000 and 10 000 pmol/egg (Quilley & Rifkind, 1986).

7.4.8.8 Studies on cell cultures

TCDD has a very low toxicity in cell cultures, yet it is a very potent inducer of AHH activity in these systems, including lymphocytes and primary hepatocytes, as well as established and transformed cell lines.

The inducibility of lymphocyte AHH has been investigated in mitogen-stimulated human lymphocytes from the venous blood of healthy volunteers. Kouri et al. (1974) found a dose-dependent increase in AHH activity (0, 0.1, 1.0, 10, or 100 ng TCDD/ml medium for 24 h). The optimal dose was about 10 ng/ml, and the maximal induction was by a factor of 2 to 3. On the contrary, Gurtoo et al. (1979) found no

dose-response correlation, in the dose range 1.7 to 20 ng TCDD/ml,
when measuring lymphocyte AHH induction. To circumvent the limitation
of prior mitogen activation when studying AHH induction in
lymphocytes, Freedman et al. (1979) used the human B-lymphocyte
RPMI-1788 cell line, which does not require prior activation for the
induction of AHH activity. The optimal concentration to stimulate AHH
activity was determined to be 10 ng/ml medium. Highly variable
induction of AHH (between 3- and 28- fold) was obtained by Nagayama et
al. (1985b) in human lympho-blastoid cell lines derived from the
peripheral blood of healthy volunteers of both sexes and of variable
ages. The cells were exposed to 7.5 ng TCDD/ml medium for 48 h.

     In a study by Niwa et al. (1975), the estimated ED50 values for
AHH induction by TCDD in 11 established cell lines, in fetal primary
cultures from five animal species and cultured human lymphocytes,
ranged from 0.04 ng/ml medium in C57Bl/6 mouse cultures and 0.08 ng/ml
in the rat hepatoma H-4-IIE cell line to more than 66 ng/ml in the HTC
rat hepatoma cell line. TCDD was demonstrated to be the most potent
AHH inducer out of 24 chlorinated dibenzo-p-dioxin analogues
(Bradlaw et al., 1980) tested in a rat hepatoma cell culture extremely
sensitive to AHH induction, the ED50 being about 0.5 pg/106 cells.
A 165-fold increase in AHH-activity and a 54-fold increase in EROD
activity were obtained in rat hepatoma H-4-IIE cells when exposed to
2×10^{-10} mol TCDD/litre for 3 days (Keys et al., 1986). In this
system co-exposure of TCDD with 1,3,6,8-tetraCDF and 2,4,6,8-tetraCDF
reduced the TCDD-induced enzyme induction, whereas co-exposure of TCDD
and TCDF resulted in an additive effect on enzyme induction (Keys et
al., 1986). The EC_{50} values for AHH and EROD induction in the same
cell system varied over 7 orders of magnitude for 14 different PCDDs
(Table 56), the most potent being TCDD and the least potent being
2,3,6-triCDD (Mason et al., 1986). A 2-to 650-fold AHH induction was
observable in 8 of 22 different cell cultures exposed to 10^{-9} mol
TCDD/litre for 24 h (Knutson & Poland, 1980a). The cells were derived
from tissues and/or species susceptible to TCDD toxicity in vivo.
Nanomolar concentrations of TCDD induced AHH activity in keratinocyte
cultures of human (Willey et al., 1984) and animal origin (Knutson &
Poland, 1980a).

     Five human squamous carcinoma cell lines derived from tumours of
the epidermis and tongue responded to TCDD with increased
O-deethylase activity, the EC_{50} being 10^{-10} to 10^{-9} mol/litre
(Hudson et al., 1983a).

     Steward & Byard (1981) treated primary hepatocytes isolated from
Sprague Dawley rats, for 48 h with various concentrations of TCDD.

They found a 2-fold induction of the AHH activity with 3 pg TCDD/106 cells. Maximal induction occurred with 2.4 ng TCDD/106 cells. Primary hepatocytes, isolated from adult male Wistar rats, exhibited a linear increase (from 2-to 4-fold) in AHH activity when exposed to TCDD in the range 10^{-11} to 10^{-8} mol/litre for 72 h (Jansing & Shain, 1985). Primary hepatocytes from TCDD-treated (5, 10, or 25 µg TCDD/kg body weight) rats, isolated 2 to 30 days post-treatment, showed decreased ouabain and alpha-aminoisobutyric acid uptake as well as tyrosine aminotransferase activity (Yang et al., 1983a). Treatment of rats with 25 mg 1,3,6,8-tetraCDD/kg body weight did not affect these parameters. Neither could these effects be demonstrated in primary hepatocytes from control rats that were treated with TCDD (50, 100, or 200 nmol/litre medium) in vitro for 48 h.

The induction of AHH and EROD activities of a complex PCDD/PCDF mixture from a fly ash extract has been reported (Safe et al., 1987).

7.4.9 Endocrine effects

Human exposure to TCDD has resulted in hirsutism and chloracne (Table 64), symptoms that suggest an alteration in endocrine regulation. Furthermore, chronic exposure to TCDD impaired reproduction in experimental animals, possibly by interfering with the estrous cycle (Kociba et al., 1976; Allen et al., 1977; Barsotti et al., 1979; Murray et al., 1979). The ability of TCDD to mimic natural steroids with steroid-like actions has prompted studies on the binding of TCDD to steroid hormone receptors.

Over-production of glucocorticoids mimics some of the symptoms of TCDD toxicity, e.g., involution of lymphoid tissues, oedema, and mobilization of fatty acids from adipose tissues. Thus TCDD might increase glucocorticoid activity by binding to glucocorticoid receptors. However, TCDD was unable to displace ^{3}H-dexamethasone, a potent synthetic glucocorticoid, from the normal rat cytosol glucocorticoid receptor even when present in 200-fold molar excess (Neal et al., 1979). Poland et al. (1976) demonstrated that cortisol and synthetic glucocorticoids did not bind to the TCDD receptor. An increase in the plasma level of corticosterone was found in male Sprague Dawley rats 7 and 14 days after a single oral dose of 50 µg TCDD/kg body weight (Neal et al., 1979). With the method used, a variety of fluorescent adrenocortical steroid hormone derivatives was measured. In contrast, Balk & Piper (1984), using a competitive binding radioassay for corticosterone, reported decreased blood levels

(29 and 26% of controls) of corticosterone in male Sprague Dawley rats on days 14 and 21, respectively, after a single oral dose of 25 µg

TCDD/kg body weight. Accumulation of 11-ß-hydroxy-progesterone in the blood of TCDD-treated rats was noticed on day 14 (Balk & Piper, 1984). Neal et al. (1979) reported 100% mortality within 6 days in adrenalectomized rats given 10, 20, 40, or 80 µg TCDD/kg body weight. Adrenalectomy and hypophysectomy could not prevent liver lesions, reduced growth rate, or thymic involution in female Fisher-344 rats given a single oral dose of 10 or 20 µg TCDD/kg body weight (van Logten et al., 1980). Thymic effects of TCDD became even more severe after hypophysectomy. Daily sc injections of 0.25 mg growth hormone had a positive influence on body weight gain but did not protect against thymic involution in hypophysectomized rats.

Single oral or interperitoneal doses of TCDD between 7 and 100 µg/kg body weight decreased the serum thyroxine (T4) level in the rat (Bastomsky, 1977; Potter et al., 1983, 1986b; McKinney et al., 1985; Pazdernik & Rozman, 1985; Rozman et al., 1985b), but not in the guinea-pig, after a single oral dose of 2 µg/kg body weight (McKinney et al., 1985). The serum triiodothyronine (T3) level in TCDD-treated rats was reported to be increased (Bastomsky, 1977; Potter et al., 1986b), unaffected (Potter et al., 1983), or decreased (Pazdernik & Rozman, 1985; Rozman et al., 1985b). Increased serum thyrotropin (TSH) (Bastomsky, 1977; Potter et al., 1986b), increased thyroid ^{131}I-uptake and increased biliary excretion of T4, but not of T3 (Bastomsky, 1977) have been reported in TCDD-treated rats.

Rats treated with TCDD at doses up to about 30 mg TCDD/kg body weight and pair-fed controls had similar serum T4, T3, and TSH levels when compared to ad libitum fed control rats (Potter et al., 1983, 1986b). Thus, it is unlikely that hypophagia is responsible for the TCDD-induced changes in serum thyroid hormone levels. When mature TCDD-treated rats were compared to pair-fed controls, there were no functional alterations in thyroid status or thermogenesis, including increased serum levels of T_3, T_4, and TSH after acute cold challenge, increased total oxygen consumption after moderate cold exposure or decreased basal metabolic rate as compared to ad libitum fed control rats (Potter et al., 1986b). A significant hypothermia was, however, observed in young TCDD-exposed rats receiving 45 µg/kg body weight as a single ip dose (Potter et al., 1983).

Available data on serum T_4, T_3, and TSH levels are not sufficient to state whether TCDD-treated rats are functionally hypothyroid, euthyroid, or hyperthyroid.

In ovo exposure of white Leghorn chicken embryos to TCDD in

the dose range 1 to 10 000 pmol/egg increased the cardiac release of prostaglandins (Quilley & Rifkind, 1986). Potter et al. (1983) reported decreased levels of insulin in serum and pancreas and of

somatostatin in the gastric antrum of Sprague Dawley rats 7 days after a single ip dose of 45 µg TCDD/kg body weight, when compared to pair-fed control rats. The somatostatin levels in serum, liver, and pancreas were not affected, neither was the serum glucagon level.

The finding that steroids are an endogenous substrate for the hepatic MFO system (Kuntzman et al., 1965) suggests that compounds, such as TCDD, that influence the activity of this enzyme system may alter steroid metabolism in vivo, and consequently also the magnitude of steroid-mediated functions. As demonstrated by Gustafsson & Ingelman-Sundberg (1979), the metabolic profiles of 4-androstene-3, 17-dione, 5 alpha-androstane-3 alpha, 17 ß-diol and 4-pregnene-3, 20-dione in hepatic microsomes from SD rats, treated with 20 µg TCDD/kg body weight for 4 consecutive days, were changed when compared to control rats 1 day post-treatment. The changes were most pronounced in female rats. When five daily doses of 1 µg TCDD/kg body weight were given to pregnant rats for 12 or 13 days during gestation, hepatic microsomes showed decreased ability to form catechol estrogens and to hydroxylate testosterone. However, this decrease did not relate to altered circulating estradiol levels (Shiverick & Muther, 1983). TCDD treatment did not affect the glucuronidation of testosterone and estrogen (Lucier et al., 1975a) or prostaglandin synthesis (Kohli & Goldstein, 1981). TCDD decreased the hepatic and uterine estrogen receptor levels in 25-day-old Long-Evans rats 2 to 10 days after treatment with single ip doses of 20 or 80 µg/kg body weight (Romkes et al., 1987). Decreased estrogen receptor levels in the liver and uterus also occurred 2 days after treatment with 1,2,7,8-tetraCDD, 1,2,3,7,8- pentaCDD, and 1,2,4,7,8-pentaCDD, but dose-response relationships were present only for TCDD and 1,2,3,7,8-pentaCDD. The estradiol-induced increases in hepatic and uterine estrogen receptor levels were counteracted by simultaneous TCDD treatment, although the effect of TCDD was not dose dependent. Hepatic hydroxylation of testosterone in 2 ß-and 16 alpha- positions was not affected in male Sprague Dawley rats (190-200 g) 3 days prior to a single oral dose of 15 µg TCDD/kg body weight (Hook et al., 1975b). Somewhat younger male Wistar rats (100 g) treated with a single dose of 0.06 mmol TCDD/kg body weight exhibited increased levels of 7 alpha-hydroxytestosterone and decreased levels of 3alpha-, 16alpha- and 16 ß-hydroxylated testosterone, as well as of androstenedione, in hepatic microsomes when compared to control rats (Keys et al., 1985). Decreased cytochrome P-450 content was found in guinea-pig (Tofilon, 1980) and rat (Tofilon & Piper, 1982) testes for at least one week after oral

TCDD treatment of 1 µg/kg and 25 µg/kg body weight, respectively. Testicular AHH activity was induced by TCDD in mice (Mattison & Thorgeirsson, 1978), but not in rats after a single oral dose of 20 µg TCDD/kg body weight (Aitio & Parkki, 1978). Mittler et al. (1984) studied the effect of single ip doses of 0.2, 1, or 5 µg TCDD/kg body weight on testicular 16-alpha-testosterone hydroxylase (16-TH), 6-ß-hydroxytestosterone (6-HT), and 7-alpha-hydroxy testosterone (7-HT) activities in young Sprague Dawley rats 90 h after exposure.

Seminiferus 16-TH activity was increased from a non-detectable level to 0.07-0.14 pg/mg protein and interstitial 6-HT activity was increased 4-fold in TCDD-treated animals. The 7-HT activity was not affected by TCDD, neither in the seminiferous tubules nor in the interstitial fraction. Serum testosterone and dihydrotestosterone were depressed dose-dependently by TCDD treatment, the ED_{50}s being about 15 µg/kg body weight in male Sprague Dawley rats, when compared to pair-fed and ad libitum fed controls (Moore et al., 1985). The plasma clearance and biliary excretion of ^{3}H-testosterone was not affected in Sprague Dawley rats treated with 100 µg TCDD/kg body weight before an i.v. injection of ^{3}H-testosterone, neither did castrated rats with implanted testosterone-leaking capsules respond to a dose of 15 or 100 µg TCDD/kg body weight with decreased accessory sex organ weights (Moore & Peterson, 1985). Increased AHH and O-deethylase activities and cytochrome P-450 content have been reported in rat prostate after single ip and oral doses, respectively, of 10 µg/TCDD/kg body weight (Haaparanta et al., 1983; Lee & Suzuki, 1980).

7.4.10 Vitamin A storage

     Decreased hepatic vitamin A storage has been reported in animals exposed to various chlorinated aromatic compounds (Table 57). Compared to other chlorinated hydrocarbons for which this effect has been evaluated, TCDD is more potent in its ability to reduce the vitamin A content of the liver.

     A single oral dose of 10 µg TCDD/kg body weight decreased both the total amount and the concentration of vitamin A in the liver of adult male Sprague Dawley rats (Thunberg et al., 1979). The decrease was evident 4 days after dosing and progressed with time. After 8 weeks the treated animals had a total liver vitamin A content corresponding to 33% of that of controls. Decreased dietary intake of vitamin A could not account for this difference. In a four-week study TCDD was given as a single oral dose of 0, 0.1, 1.0, or 10 µg per kg body weight to adult male Sprague Dawley rats fed ad libitum with

pelleted diets containing 1.2 (low), 3.0 (normal), or 6.0 (high) mg
vitamin A/kg diet (Thunberg et al, 1980). Both the concentration and
the total amount of vitamin A were decreased in a dose-dependent
manner in the animals receiving the high vitamin A diet. In the
animals on the normal and low vitamin A diets, significant differences
were seen only at doses of 1.0 and 10 µg TCDD/kg body weight. A
significant increase in the UDPGT activity was observed in all dietary
groups treated with 1.0 and 10 µg TCDD per kg body weight, suggestive
of an increased excretion of vitamin A conjugated with glucuronic acid
(Thunberg & Hakansson, 1983). However, no correlation between the
UDPGT activity and the reduction of hepatic vitamin A levels was seen
when homozygous Gunn rats lacking inducible UDPGT were treated with a
single oral dose of 20 µg/kg body weight (Aitio et al., 1979) nor in
heterozygous Gunn rats with inducible UDPGT after a single oral dose
of 10 µg/kg body weight (Thunberg & Hakansson, 1983).

Table 57. The potency of various chlorinated cyclic hydrocarbons to
reduce hepatic vitamin A content in the rat

Compound (Reference) route of administration	Strain/sex/age Duration of the study	% Reduction of hepatic vitamin A	Dose and
Arochlor 1242 in diet[a] (Cecil et al., 1973)	Rattus norvegicus/M,F[f]/21 days 2 months	49	100 mg/kg
p,p-DDT in diet[a] (Cecil et al., 1973)	Rattus norvegicus/M,F/21 days 2 months	38	100 mg/kg
Methoxychlor in diet[b] (Davison & Cox, 1976)	Sprague Dawley/NR[f]/23 days 16 weeks	7	10 mg/kg
in diet		12	100 mg/kg
in diet		37	1000 mg/kg
in diet		68	10 000 mg/kg

PCB in diet[c] (Innami et al., 1976)	Sprague Dawley/M/21 days 8 weeks	100 mg/kg 82	

TCDD kg bw[f] (Thunberg et al., 1979) (single oral dose)	Sprague Dawley/M/NR 7 days 14 days	10 µg/ 29 39	
days		59	28
days		67	56

Table 57 (contd).

Compound route of (Reference) administration	Strain/sex/age Duration of the study	Dose and % Reduction of hepatic	
			vitamin A

TCDD kg bw[g] (Thunberg et al., kg bw[g] 1980) kg bw[g]	Sprague Dawley/M/NR 28 days	0.1 µg/ 2 1.0 µg/ 27 10.0 µg/ 65	

TCDD kg bw[g] (Hakansson, 1988) kg bw[g] kg bw[g] kg bw[g]	Sprague Dawley/M/2 months 44 days	15 µg/ 59[d] 88[e] 30 µg/ 78[d] 98[e] 60 µg/ 81[d] 97[e] 120 µg/ 90[d] 99[e]	

Toxaphene kg bw[h] (Thunberg et al., 1984)	Sprague Dawley/M/NR 4 weeks	20 mg/ 0	

a   9-12 mg vitamin A/kg diet <u>ad</u> <u>libitum</u>.
b   33 000 IU vitamin A/kg diet <u>ad</u> <u>libitum</u>.
c   3000 IU vitamin A/kg diet <u>ad</u> <u>libitum</u>.
d   21 000 IU vitamin A/kg diet <u>ad</u> <u>libitum</u>.
e   8000 IU vitamin A/kg diet <u>ad</u> <u>libitum</u>.
f   M = male; F = female; NR = not reported; bw = body weight.
g   Single oral doses.
h   Orally twice weekly.

     Male Sprague Dawley rats received a single oral dose of 10 µg
TCDD/kg body weight 4 days prior to the oral administration of a
single physiological dose of labelled vitamin A,
(11,12-^{3}H)retinylacetate (RA) (Hakansson & Ahlborg, 1985a). The
distribution and elimination of the radiolabel, and the vitamin A
content in various tissues were determined 1, 6, 12, 24, 72, and 192
h after the administration of ^{3}H-vitamin A. The body burden of
radioactivity remained around 40% of the administered dose in control
rats throughout the study, whereas in TCDD-pretreated rats the body
burden decreased continuously from 21% at 12 h after administration to
11% at the end of the study. More of the radiolabel was recovered in
the kidney, testes, epididymis, and serum of TCDD-treated animals than
in controls, when calculated as percentage of body burden, whereas
less was recovered in the liver. Forty percent of the dose was
eliminated via faeces and urine in TCDD-treated rats, compared to 23%
in controls. More radioactivity was eliminated in faeces than in urine
both in control and TCDD-pretreated animals, although urinary
elimination was more pronounced in TCDD-pretreated than in control
rats. It was concluded that TCDD-treated rats handled the newly
administered dose of vitamin A in a similar way to rats deficient in
Vitamin A (Huque, 1981; Blomhoff et al., 1982). This finding is
remarkable since the TCDD-treated animals in this study still had
considerable stores of hepatic vitamin A, and did not show decreased
levels of serum vitamin A, i.e., they were not deficient in vitamin A.
In a similarly designed study the effect of a single oral dose of 10
µg TCDD/kg body weight on the endogenous pool of vitamin A
(radiolabelled 15-^{3}H-retinol given 5 to 7 days prior to TCDD
treatment) was studied in Sprague Dawley rats with stores of low liver
vitamin A (Hakansson et al., 1986). It was demonstrated that
endogenously stored vitamin A was rapidly depleted from the liver of
TCDD-treated rats and was eliminated both in faeces and in urine. An

increased distribution of the vitamin A stored in the liver to extrahepatic tissues was also seen in the treated rats.

To elucidate whether dietary vitamin A would reduce TCDD toxicity, Hakansson (1988) fed male Sprague Dawley rats ad libitum from weaning throughout the experiment with diets containing 2000 (I), 5000 (II), 8000 (III), or 21 000 (IV) IU of vitamin A/kg. A single oral dose of TCDD (15, 30, 60, or 120 µg TCDD/kg body weight) was given when the rats were 8 weeks old and the animals were killed 44 days post-treatment. With diet IV, TCDD reduced in a dose-dependent manner hepatic vitamin A by 59 to 90%. With diets II and III, reduction of hepatic vitamin A was more than 95% after dosing with 15 and 30 µg TCDD/kg, respectively. In control animals fed diet I, total hepatic vitamin A was less than 1 µg and TCDD had no further effect at any dose. Serum vitamin A was dose-dependently decreased by TCDD treatment in dietary groups I, II, and III, whereas in dietary group IV TCDD increased serum vitamin A. Only with the highest TCDD dose was there a counteraction by dietary vitamin A on all of the above parameters.

A single dose of 10 µg TCDD/kg body weight to female Sprague Dawley rats on the day of delivery affected the vitamin A content in the liver and kidney of the offspring (Hakansson et al., 1987). The effect became clearly visible after weaning, i.e., when the dietary intake of vitamin A was high enough to allow for storage. At the end of the study (postnatal day 32), the vitamin A content in the liver was 225 µg in control pups and 102 µg in TCDD-exposed pups. The corresponding values for the kidney vitamin A content were 1.4 and 8.4 µg, respectively. The TCDD-induced effects on vitamin A levels in the liver and kidneys followed a similar time-course as the growth reduction, i.e., a minor effect throughout the lactation period, which became more pronounced post-weaning. This was in contrast to the liver enlargement and thymus involution in TCDD-exposed pups, which were most pronounced throughout lactation and tended to diminish post-weaning.

Single oral doses of 0, 1, 5 and 10 µg TCDD/kg body weight or a mixture of PCDDs and PCDFs, reconstituting the levels found in human milk, were given to male Sprague Dawley rats (80 g) in corn oil (Ahlborg et al., 1987). The mixture was given at three dose levels resulting in 1, 5, or 10 µg TCDD/kg body weight. Four weeks after dosing there was a dose-dependent decrease in hepatic vitamin A in TCDD-treated rats; no further effect was seen in the animals treated with PCDD/PCDF mixtures. In contrast, mixture treatment had an additive effect, as compared to TCDD alone, on the increases in renal vitamin A and hepatic cytochrome P-450 contents, AHH activity, and

UDPGT activity.

Taken together these data indicate that TCDD interferes with the storage mechanism for vitamin A. In the liver this mechanism has been thoroughly investigated (Hirosawa & Yamada, 1973; Blomhoff et al., 1982; Olson & Gunning, 1983). As dietary vitamin A seems unable to counteract all toxic effects, this would imply either that the effect on vitamin A storage is secondary to TCDD toxicity or that the cellular utilization of vitamin A is affected by TCDD.

7.5 Embryotoxicity and Reproductive Effects

The teratogenic potential of TCDD was first demonstrated in rats and mice by Courtney & Moore (1971). This followed the finding that 2,4,5-trichlorophenoxyacetic acid contaminated with 30 mg TCDD/kg was teratogenic in two strains of mice and one strain of rat (Courtney et al., 1970), leading to an increased incidence of cleft palate and cystic kidney in both strains of mice and cystic kidney in rats. Further studies have revealed that TCDD is fetotoxic rather than teratogenic in the rat, producing subcutaneous oedema, haemorrhages, and slight kidney anomalies (see section 7.5.1). In contrast, TCDD produces a specific teratogenic response, consisting of cleft palate and kidney malformations, in several strains of mice (see section 7.5.2 and Table 58). Extra ribs, minor abnormalities in the palate,

and cardiovascular malformations have been demonstrated in rabbits, monkeys, and chickens, respectively, after exposure to TCDD in utero (see sections 8.5.3-8.5.6). Impaired reproductive performance due to TCDD exposure has been demonstrated in rats and monkeys (see sections 8.5.1 and 8.5.4).

7.5.1 Studies on rats

The initial studies on the embryotoxic effects of TCDD in the rat were performed with Charles River CD (Courtney & Moore, 1971), Sprague Dawley (Sparschu et al., 1971), and Wistar rats (Khera & Ruddick, 1973). In these studies maternal toxicity was noted at doses > 0.5 (Sparschu et al., 1971) or 1 µg/kg body weight per day (Khera & Ruddick, 1973). Decreases in gestational survival, fetal weight, and postnatal survival were reported at dose levels in the range 0.125-0.5 µg TCDD/kg body weight per day on gestation days 6-15 or 5-14. Haemorrhages, mainly intestinal, and subcutaneous oedema were common findings at similar doses. The teratogenic findings were limited to kidney anomalies, described as unilocular cystinephrotic kidney or as hydronephrosis, in the CD rat at or above 0.5 µg TCDD/kg per day (Courtney & Moore, 1971). Slightly dilated renal pelvis was reported

in the F1 generation at the 0.01 µg/kg per day dose level in a three-generation reproductive study of TCDD in Sprague Dawley rats (Murray et al., 1979). Giavini et al. (1983) found an increased incidence of renal anomalies in Charles River CD offspring exposed to 2 µg TCDD/kg body weight on gestation days 0 to 2, but not in Sprague Dawley offspring when the dam was given daily oral doses of 0, 0.125, 0.5, and 2 µg TCDD/kg body weight for 2 weeks before mating (Giavini et al., 1982a).

Sparschu et al. (1971) found an increased number of resorption sites in Sprague Dawley rats at doses $\geq$ 0.5 g TCDD/kg per day, but no effect on ovulation rate and preimplantation loss was reported even at 2 µg/kg per day. TCDD exposure (0.1, 0.5, or 2.0 µg/kg per day) on gestation days 0 to 2 had no effect on the reproductive performance of Sprague Dawley rats (Giavini et al., 1983). In contrast, impaired reproductive performance was found in Charles River CD rats after receiving TCDD for two weeks before mating (Giavini et al., 1982a). The number of resorption sites was elevated at doses of 0.5 µg TCDD/kg per day or more, whereas the ovulation rate and preimplantation loss was affected only at 2 µg/kg per day.

The male reproductive ability was affected by TCDD treatment only at toxic doses, as judged by studies of Khera & Ruddick (1973) and Murray et al. (1979). Nevertheless, decreased mating frequency was noted in the groups, of the F_0 generation that received 0.1 µg TCDD/kg per day in the diet (Murray et al., 1979). No effect on the mating frequency was found by Giavini et al. (1982a, 1983).

In a three-generation reproduction study, Murray et al. (1979) maintained Sprague Dawley rats on diets providing doses of 0, 0.001, 0.01, or 0.1 µg TCDD/kg body weight per day. The F_0 generation received the diet for 90 days before mating. No toxic effects were observed in the F_0 generation but decreased body weight and reduced food consumption were noted in the F_1 and F_2 generations at 0.01 µg/kg per day. Fertility was greatly reduced at 0.1 µg/kg per day in the F_0 generation. This group was discontinued because of the low number of offspring. In the F_1 and F_2 generations fertility was significantly reduced at 0.001 and 0.01 µg/kg per day, respectively. At 0.01 µg/kg per day, litter sizes were reduced, and fetal and neonatal survival were decreased as well as postnatal growth. Murray et al. (1979) concluded that doses of 0.1 and 0.01 µg/kg per day impaired reproduction in rats. A dose of 0.001 µg/kg per day had no effect on fertility, litter size, postnatal body weight, or neonatal

survival and was therefore suggested to be a no-effect dose for
reproductive lesions.

Reevaluation of these data by Murray et al. (1979) using another
statistical model, including pooling of the data from the four
generations, led to the conclusion that the 0.001 µg/kg per day dose
level did affect reproduction and thus was not a no-effect level, but
a low-effect level (Nisbet & Paxton, 1982). Kimbrough et al. (1984)
considered that the data by Murray et al. (1979) could not be used for
risk assessment calculations due to the great variation in fertility
index both in controls and exposed rats.

No embryotoxic and/or reproductive effects were found when female
Wistar rats were exposed to 1,2,3,4-tetraCDD (50, 100, 200, 400, or
800 µg/kg body weight per day), 2,7-diCDD (250, 500, 1000 or 2000
µg/kg body weight per day); 2,3-diCDD (1000 or 2000 µg/kg body weight
per day), or 2-monoCDD (1000 or 2000 µg/kg body weight per day) on
gestation days 6-15 (Khera & Ruddick, 1973). The maturation process of
the lung was not affected in Sherman rats exposed to 2,7-diCDD (40
µg/kg per day on gestation days 7-15) (Kimbrough et al., 1974).

7.5.2 Studies on mice

TCDD-induced embryo mortality in NMRI mice was significantly
increased at doses of 4.5 and 9 µg TCDD/kg body weight per day when
given on gestation days 6-15, but no embryotoxic effect was observed
when daily doses of 9 µg/kg body weight were given on gestation days
9-13 (Neubert & Dillman, 1982). The number of resorptions on day 13 of
gestation in NMRI mice was increased, as compared to controls, when 25
µg TCDD/kg body weight was given, divided into five daily doses on
days 7-11 of gestation, but no effect was observed when the same dose
was given as single ip injections on days 7 or 10 of gestation (Nau &
Bass, 1981). A single ip dose of 30 µg TCDD/kg body weight on
gestation day 11 had no effect on the fetal mortality in C57Bl/6 mice
on days 12, 13, or 14 of gestation (Weber & Birnbaum, 1985). Decreased
pre- and postnatal survival rates and retarded postnatal development
were observed in NMRI mice given four oral doses of 12.5 µg TCDD/kg
body weight on gestation days 14-17 (Nau et al., 1986). The cumulative

mortality was 45%, 68.5%, and 75% in exposed offspring on postnatal
days 1, 14, and 22, respectively, as compared to 6% in controls on day
22.

Table 58 summarizes the early studies on the teratogenic effects
of TCDD in various strains of mice. These studies (Courtney & Moore,
1971; Neubert & Dillman, 1972; Courtney, 1976; Smith et al., 1976),

revealed that TCDD is a specific teratogen in mice causing increased frequencies of kidney anomalies and cleft palate at doses well below those which result in fetal mortality and maternal toxicity.

The TCDD-induced kidney anomaly is morphologically described as a progressive hydronephrosis, preferentially occurring in the right kidney, and never accompanied by hydroureter or abnormal nephron development (Courtney & Moore, 1971; Moore et al., 1973; Birnbaum et al., 1985; Weber et al., 1985).

The fetal kidney seems to be more susceptible to TCDD exposure than the developing palate (Courtney & Moore, 1971; Moore et al., 1973; Birnbaum et al., 1986; Weber et al.,1984). The incidence of kidney anomalies after a single dose of 1 µg TCDD/kg body weight on gestation day 10 in C57Bl/6 mice was 34.3%. If the same dose was divided and given on gestation days 10-13, the incidence of kidney anomalies was 58.9%. The incidences of cleft palate in the same studies were 0 and 1.9%, respectively (Moore et al., 1973). In contrast, in the CF-1 strain of mice, cleft palate was a more sensitive parameter than hydronephrosis, occurring at 1 and 3 µg TCDD/kg body weight per day on gestation days 6 to 15, respectively (Smith et al., 1976). It would appear that 3 µg TCDD/kg per day on gestation days 10-13 is close to a threshold dose for cleft palate induction in C57Bl/6 mice (Moore et al., 1973; Birnbaum et al., 1986). The maximum increase in cleft palate incidence is produced if TCDD is administered on any individual day from gestation day 8-10 in C57Bl/6 mice (Pratt et al., 1984) or on gestation day 10-11 in NMRI mice (Nau & Bass, 1981; Neubert & Dillman, 1982; Krowke, 1986). The cleft palate incidence in C57Bl/6 mice was more pronounced (36%) when TCDD was given as a single oral dose of 12 µg/kg body weight on gestation day 11 than if 3 µg/kg body weight per day was given on gestation days 10-13 (Birnbaum et al., 1985). No difference was seen in the incidence of kidney anomalies with the same treatment. TCDD is not a potent inducer of cleft palate when given on day 13 of gestation or later (Neubert & Dillman, 1982; Pratt et al., 1984). Examination of cryostat sections taken from C57Bl/6 embryos during the time of palatal elevation and fusion demonstrated that TCDD does not interfere with growth, elevation, or initial contact of the palatal shelves but does interfere with the firm adhesion and/or degeneration of the medial epithelial cells, i.e., programmed epithelial cell death does not appear to occur in the medial epithelium in embryos exposed to TCDD (Pratt et al., 1984). The cleft palates that were observed were complete clefts of the entire hard and soft palate and no clefts of the primary palate were observed (Pratt et al., 1984).

Table 58. Early studies on embryotoxic effects of TCDD in mice

Strain/sex[b] Parental toxicity (reference)	Route/vehicle/dose (μg/kg body weight day)	Treatment/ observation (days)[a] Embryotoxic effects
CD-1/F No effect (Courtney & DMSO/1.0, 3.0 anomalies at doses ≥ 1 μg/kg Moore, 1971) per day.	subcutaneous/	6-15 / 18 Increased incidence of kidney
BDA/2J Increased relative (Courtney & liver weight Moore, 1971)	subcutaneous/ DMSO/3.0	6-15 / 17 Increased incidence of cleft palate and kidney anomalies.
C56BL/6J Increased relative (Courtney & liver weight Moore, 1971)	subcutaneous/ DMSO/3.0	6-15 / 17 Increased incidence of cleft palate and kidney anomalies.
NMRI/F None reported (Neubert & 3.0, 4.5, per day. Increased Dillman, 1972) 9.0 incidence of cleft palate at ≥ 3 μg/kg per day.	oral/rape seed oil/0.3,	6-15 / 18 Increased number of resorptions at 9 μg/kg doses
C56BL/6J None reported	oral/corn oil:acetone	10-13 / 18 Increased incidence of cleft

      (Neubert &
(9:1)/1.0, 3.0   palate
at doses $\geq$ 3 µg/kg
      Dillman,
1972)
per day increased incidence

                                                                                        of

kidney anomalies at doses

                                                                                        $\geq$

1 µg/kg per day.

      Table 58 (contd).

      Strain/sex[b]   Route/vehicle/dose   Treatment/
Parental toxicity   Embryotoxic effects
     (reference)   (µg/kg body weight   observation
                                day)   (days)[a]

      CD-1/F   oral/corn oil:anisole   7-16 / 18
Increased maternal   Increased fetal mortality at all
     (Courtney, 1976)   (95:5)/25, 50, 100,   relative
liver weight   doses. Increased incidence of
                                200, 400   at 25 and 50
µg/kg per   kidney anomalies and cleft palate

                                                                         day.
Marked oedema and   at doses $\geq$ 25 µg/kg per day

                                                                         vaginal
bleeding at   and 50 µg/kg per day,

                                                                         doses $\geq$ 200
µg/kg per   respectively. Increased incidence

                                                                         day.
of club foot was found in the

                                                                         high-
dose group. Hydrocephalus and

                                                                         open
eyes were seen occasionally.

      CF-1   oral/corn oil:acetone   6-15 / 18
Increased maternal   Increased number of resorptions
     (Smith et al.,   (98:2)/0.001, 0.01,   relative
liver weight   at 1 µg/kg per day. Increased
      1976)   0.1, 1.0, 3.0   at 3 µg/kg

per day.   incidence of cleft palate at

doses

$\geq$ 1 µg/kg per day.

Dilated

renal pelvis occurred at

3

µg/kg per day.

---

 a First day of gestation designated day zero.
 b F = female.

     Poland & Glover (1980) reported that in nine out of ten inbred
strains of mice, the susceptibility to cleft palate formation produced
by TCDD (30 µg TCDD/kg body weight sc on day 10 of pregnancy) followed
the distribution of the Ah locus within that strain. The five strains
with a low affinity TCDD receptor in the liver (DBA/2, RI, AKR, SWR,
and 129) developed cleft palate at an incidence of 0 to 3% while four
of the five strains with a high affinity TCDD receptor in the liver
(C57Bl/6, A, BALB/cBy, and SEC) developed cleft palate at an incidence
of 54 to 95%. The only strain with a high affinity hepatic TCDD
receptor that did not develop cleft palate was CBA. Mid-gestational
mice embryos from C57Bl/6 exhibit high levels of the TCDD receptor in
the maxillary processes and secondary palatal shelves whereas no
specific binding of TCDD could be demonstrated in the AKR strain
(Dencker & Pratt, 1981). Evidence that TCDD interferes in embryonic
development by directly interacting with embryonic cells rather than
being secondary to maternal effects was presented by D'Argy et al.,
(1984) in a study where mouse blastocysts were transplanted between
NMRI (sensitive) and DBA (non-sensitive) dams on gestation day 3. TCDD
treatment (30 µg/kg body weight) on gestation day 10 resulted in 75 to
100% incidence of cleft palate among NMRI fetuses whether they
remained in their own dams or as aliens in DBA dams. Also, none of the
DBA fetuses developed cleft palate whether or not they remained in
their own dams or as aliens in NMRI dams.

     Attempts to modify and further characterize the TCDD-induced
cleft palate formation have been performed with several interacting
substances. The non-teratogenic ß-naphthoflavone (ßN) enhanced the
TCDD-induced fetal mortality and the increase in the incidence of
cleft palate in C57Bl/6 and NMRI mice when ßN was administered
simultaneously or 8 h before TCDD, but not 24 h before or after

(Hassoun & Dencker, 1982). TCDD was given as a single ip dose of 25 or
16 mg/kg body weight to C57Bl/6 and NMRI mice, respectively, on
gestation days 10, 11, 12, or 13. TCDD-induced cleft palate incidence
was enhanced by 2,3,4,5,3',4'-hexachlorobiphenyl, but not
2,4,5,2',4',5'-hexachlorobiphenyl, in C57Bl/6 mice, although none of
the isomers alone were teratogenic at the doses used (Birnbaum et al.,
1985). Neither of the hexachlorobiphenyls used affected the incidence
of renal anomalies, as compared to TCDD alone.

A dose-related enhancement of the TCDD-induced incidence of cleft
palate was found in C57Bl/6 mice exposed to either triiodothyronine or
thyroxine, as compared to TCDD alone (Lamb et al., 1986). The
hydrocortisone-induced cleft palate response was enhanced by
simultaneous administration of TCDD in C57Bl/6 mice (Birnbaum et al.,
1986). Co-administration of TCDD and TCDF to C57Bl/6 mice on gestation
day 10 resulted in a cleft palate incidence compatible with an
additive toxicity model in which TCDF contributes to the toxicity of
TCDD in a weight ratio of 1:30 (Weber et al., 1985). Also 1,2,3,7,8-
pentaCDD and 1,2,3,4,7,8-hexaCDD had additive effects on the
TCDD-induced cleft palate incidence in NMRI mice (Krowke, 1986). No

clearly dose-related teratogenic effects were observed in C57Bl/6 mice
exposed to oral doses of 1,2,3,4- tetraCDD (100, 250, 500, or 1000
mg/kg per day), octaCDD (5 or 20 mg/kg per day) or to a mixture of 40%
2,7-diCDD and 60% 2,3,7-triCDD (100 or 200 mg/kg per day) on gestation
days 7-16 (Courtney, 1976).

7.5.3 Studies on rabbits

New Zealand rabbits were administered TCDD by gavage in doses of
0, 0.1, 0.25, 0.5, and 1 µg/kg per day on days 6-15 of gestation and
the fetuses were examined on day 28 of gestation (Giavani et al.,
1982b). Above 0.25 µg/kg per day, decreased maternal weight gain and
unspecified signs of maternal toxicity were reported. At doses of 0.5
and 1 µg/kg per day, there were 2/15 and 4/10 maternal deaths,
respectively. An increase in abortion and resorption rates occurred at
doses above 0.25 µg/kg per day, with no live fetuses detected in the
1 µg per kg per day dose group. There was a significant increase in
extra ribs, compared to a level of 33.3% in the controls to 82, 66.6,
and 82%. In the 0.1, 0.25, and 0.5 µg/kg per day dose groups, 82,
66.6, and 82% extra ribs were noted. There were no increases in
specific soft tissue anomalies.

7.5.4 Studies on monkeys

The effect of exposure to TCDD in the diet, before mating and

throughout gestation, on the reproductive performance and production of progeny of healthy, fertile rhesus monkeys (<u>Macaca</u> <u>mulatta</u>) was studied by Allen and co-workers (Allen et al., 1979a,b; Barsotti et al., 1979; Schantz et al., 1979). At a level of 500 ng TCDD/kg diet (11 ng/kg body weight per day), there were no effects on the length, intensity, or duration of the menstrual cycle, but decreases in serum estradiol and progesterone levels were observed (Barsotti et al., 1979). Mating with control males at the end of the sixth month resulted in three pregnancies, out of which two resulted in abortion, and three animals failed to conceive. The remaining TCDD-treated female was not bred due to toxic symptoms. The two females that survived the study were returned to a control diet and later gave birth to well developed infants. After 7 months on the 50 ng TCDD/kg diet (1.5 ng/kg body weight per day), the reproductive outcome was: four abortions, one stillbirth, two failures to conceive and two normal births (Schantz et al., 1979).

All controls conceived and gave birth to normal infants (Barsotti et al., 1979; Schantz et al., 1979).

McNulty (1984) demonstrated that rhesus monkeys (Macaca mulatta) receiving 1 μg TCDD/kg body weight either as a single dose on gestation days 25, 30, 35, or 40, or divided into nine doses between gestation days 20-40, failed to give birth normally. Of 16 pregnancies 13 resulted in abortions. Two of the three live fetuses, obtained by Caesarean section on day 145 of gestation, showed minor abnormalities

in the palate. Maternal toxicity, manifest in 8 out of 16 females, appeared only after a period of 44-111 days after abortion. From the results obtained it was not possible to conclude whether fetal death was a direct effect of TCDD on the fetus or placenta or an indirect effect through maternal toxicity.

7.5.5 Studies on chickens

Treatment of fertile White Leghorn chicken eggs, on day 0 of development, with single doses of TCDD, ranging from 0.009 to 77.5 pmol/egg, resulted in a dose-related increase in the following types of cardiovascular malformations: ventricular septal defects, aortic arch anomalies, aortic arch anomaly plus ventricular septal defect, and conotruncal malformation (Cheung et al., 1981). The dose producing cardiovascular malformations in 50% of the embryos was about 1 pmole TCDD/egg.

A dose-dependent decrease in hatchability and increased incidences of beak, brain, and leg malformations were found in the

embryos when fertile White Leghorn eggs were injected with toxic fat
material containing 0.9, 1.8, or 4.5 ng of a mixture of dioxins (14%
diCDDs, 1% triCDDs, 38 to 45% tetraCDDs, 13% pentaCDDs, 14% hexaCDDs,
12% heptaCDDs, 8% octaCDD) (Flick et al., 1973).

7.6 Mutagenicity and Related End-Points

7.6.1 Mutagenicity

7.6.1.1 Studies on bacteria

     Results from bacterial mutagenicity tests with TCDD are
conflicting.

     In studies by Hussain et al. (1972) and Seiler (1973), a positive
response was reported in the Salmonella typhimurium strain TA 1532
without metabolic activation in a plate test after preincubation of
bacteria in medium containing TCDD and also in a spot test. A
mutagenic response was also obtained with Escherichia coli Sd-4,
measuring reversion to streptomycin independence (Hussain et al.,
1972). OctaCDD was not mutagenic to various strains of Salmonella
typhimurium (Seiler, 1973) without metabolic activation. More recent
publications, however, do not report any mutagenic effect of TCDD in
the Ames' Salmonella plate incorporation assay using strains TA
1530, TA 1532, TA 1535, TA 1537, TA 1538, TA 98, or TA 100 in the
presence or absence of metabolic activation systems from rat and
Syrian hamster liver (Gilbert et al., 1980; Geiger & Neal, 1981;
Mortelmans, 1984). In these studies the earlier reported positive
tester strain TA 1532 was either replaced by strain TA 1537 (Geiger &
Neal, 1981; Mortelmans, 1984) or was tested in addition to TA 1537
(Gilbert et al., 1980). Strain TA 1537 has been derived from TA 1532

and is more sensitive, due to its improved uptake of large molecules.
TCDD was tested in the dose range 0.2-2000 µg/plate. Due to the
limited solubility of TCDD, a maximal dose in the Salmonella system
was reported to be 20 µg TCDD/plate (Geiger & Neal, 1981).

7.6.1.2 Studies on eukaryotic cells

     Bronzetti et al. (1983) reported a mutagenic response of TCDD in
yeast in an in vitro suspension test and a host mediated assay. In
both assays Saccharomyces cerevisiae strain D7 was used. Positive
responses were obtained in vitro in the presence of metabolic
activation at doses of TCDD up to 10 µg TCDD/ml, and in the
host-mediated assay after treatment of mice with a single dose of TCDD
(25 µg/kg body weight).

In L5178Y mouse lymphoma cells, TCDD induced mutations in a dose-dependent manner at doses of 0.05-0.5 µg TCDD/ml; survival of cells at the highest concentration was at least 75% (Rogers et al., 1982).

7.6.1.3 In vivo studies

A dominant lethal test on male Wistar rats has been reported by Khera & Ruddick (1973). The rats were given 4, 8, or 12 µg TCDD/kg body weight per day orally for seven consecutive days, after which seven sequential mating trials, 5 days at a time, were conducted in the surviving males. Nine days after separation of females from the males, the females were killed. The highest dose was lethal for all males exposed (20/20); 8 µg/kg per day killed 11/20 males, and 4 µg/kg per day was lethal for 2/20. All animals in the control group survived.

The results did not indicate a dominant lethal effect during the 35 days post-treatment period, corresponding to the postmeiotic stages of spermatogenesis.

7.6.2 Interaction with nucleic acids

Poland & Glover (1979) found that very little TCDD bound to rat liver nucleic acids after treatment with 3H-TCDD in vivo; the maximum covalently bound TCDD was calculated to be 6 and 12 pmol per mol of nucleotide residues from DNA and RNA, respectively. After iv injection of ^{3}H-TCDD in rats, the radioactivity taken up by the liver cytosol decreased at the same rate as the radioactivity in the nuclear fraction increased. The radioactivity in the nuclei was at a maximum 2 h after injection (Carlstedt-Duke et al., 1982). Guenthner et al. (1979) demonstrated in vitro metabolism of TCDD to reactive intermediates that bound covalently to cellular macromolecules, principally to proteins. However, no isomer-specific methods were used for analysis of metabolites in this study.

Liver slices from Sprague Dawley rats treated with 5 µg TCDD incorporated twice the level of thymidine into nuclear DNA 10 days post-treatment than did controls (Conaway & Matsumura, 1975). Christian & Peterson (1983a) found no effect on the in vivo incorporation of thymidine into liver DNA 35 to 36 h after the administration of 10 µg TCDD/kg body weight to Sprague Dawley rats. DNA synthesis in Porton rats, stimulated by 70% hepatectomy and measured as the 1 h in vivo incorporation of thymidine, was not affected by treatment with 10 or 200 µg TCDD/kg body weight 0, 24, or

72 h before the hepatectomy was performed (Greig et al., 1974). In contrast, Dickins et al. (1981) found an 8-to 10-fold increase in DNA synthesis in response to the proliferation caused by a 1/3 hepatectomy in Sprague Dawley rats given 5 µg TCDD/kg body weight 5 days prior to the hepatectomy. In this study thymidine incorporation into DNA was measured in vitro at various times after hepatectomy. The TCDD-induced increase was most pronounced 24 to 32 h after the hepatectomy. The somewhat conflicting results may be due to differences in the in vivo and in vitro incorporation of thymidine, as well as to differences in the time points studied. According to Dickins et al. (1981), the discrepancy in proliferative DNA synthesis could be due to the degree of hepatectomy. Thus 70% hepatectomy would by itself enhance DNA synthesis to near maximum level, making it difficult to measure any effect of TCDD under the experimental conditions. This suggestion was confirmed by Christian & Peterson (1983a), who compared the effect of TCDD on proliferative DNA synthesis after 1/3 and 2/3 hepatectomy. This study also revealed that the effect could be seen only when a certain amount of time, namely 5 to 10 days, elapsed between TCDD administration and hepatectomy.

The transfectivity of bacteriophage Qß/RNA was evaluated after treatment in vitro with TCDD. No effect was noticed in the tested dose range (0.2-4 µg TCDD/ml) (Kondorosi et al., 1973).

The effect of TCDD on the repair of DNA damage induced by 2-aminofluorene (AF) and 2-acetylaminofluorene (AAF) in primary hepatocytes from B6 and D2 mice has been investigated (Moller et al., 1984). Pretreatment in vivo with TCDD (50 mmol/litre) resulted in a slight increase in DNA damage (measured by the alkaline elution technique) following incubation with either AF or AAF for 60 min, suggesting induction of aromatic amine activating enzymes.

7.6.3 Cytogenetic effects

Green & Mooreland (1975) and Loprieno et al. (1982a) did not observe any induction of chromosomal aberrations in rats administered TCDD intraperitoneally or by gavage (5 to 20 µg/kg body weight). Later Green et al. (1977) showed a significant increase in the induction of chromosomal abnormalities in bone marrow cells of male rats at doses of 2 and 4 µg TCDD/kg body weight and in females at 4 µg/kg body weight. In a study by Meyne et al. (1985) C57Bl/6 or DBA/2 mice (with

high- and low-affinity TCDD receptors, respectively) were ip injected at doses 0, 50, 100, and 150 µg/kg body weight. There was no increase in the frequency of chromosomal aberrations in bone marrow cells of the TCDD-treated mice of either strain 8, 16, or 24 h after treatment.

All doses were high enough to induce hepatotoxic damage in C57Bl/6
mice. In male and female CD-1 mice, however, a weak but significant
increase in chromosomal aberrations was obtained 96 h post-treatment
(ip 10 µg TCDD/kg body weight) (Loprieno et al., 1982b).

Lamb et al. (1981) evaluated the frequency of sister chromatid
exchange (SCE) in bone marrow cells of C57Bl/6 mice given single ip
injections of mixtures of chlorinated phenoxy acids that contained
0.16, 1.2, or 2.4 µg TCDD/kg body weight. The mean SCE frequency in
treated and in control mice did not differ significantly. Mice fed
diets containing the same daily doses as above for 4 to 8 weeks did
not show any increase in SCE frequencies.

At doses (50, 100, and 150 µg per kg body weight) hepatotoxic to
C57Bl/6 mice, there was no significant increase of SCEs in C57Bl/6 or
DBA/2 mice 18 h after ip injection of TCDD (Meyne et al., 1985). Meyne
et al. (1985) also performed a micronucleus test under the same
conditions as above. Mice, killed 24 or 48 h after treatment, did not
show any increase of micronuclei in polychromatic erythrocytes of bone
marrow in either strain.

7.6.4 Cell transformation

Single treatments (11 concentrations in the range 0 to 5
µmol/litre) of mouse embryo fibroblast (C3H/10T1/2) tissue cultures
with TCDD did not transform or initiate the process of transformation
in cultures subsequently exposed to
12-O-tetradecanoylphorbol-13-acetate (Abernethy et al., 1985).
Continued treatment of these cells with low concentrations (> 4
pmol/litre) of TCDD enhanced the production of foci in cultures
pretreated with N-methyl-N'-nitro-N-nitrosoguanidine. Maximal
enhancement occurred at 40 pmol/litre. Higher doses, 120 to 4000
pmol/litre, did not further increase the incidence of foci production.
Promotion of transformation is thus the predominant effect of TCDD in
the C3H/10T1/2 cell-transformation system.

7.7 Carcinogenicity

7.7.1 Long-term animal studies on single compounds

Several studies on the carcinogenicity of TCDD and related
compounds have been performed.

The data from studies using oral exposure are summarized in Table
59. Van Miller et al. (1977) exposed male Sprague Dawley rats to
various dietary levels of TCDD ranging from 0.001 µg/kg and 1 µg/kg to

1 µg/kg for 78 weeks. Pronounced mortality was observed at higher
doses. Neoplastic changes in different organs were noted in a number
of rats that died. At 95 weeks, the small number of surviving animals
were killed. At dietary levels of 5, 50, and 500 ppt TCDD (ng/kg
feed), a variety of tumours were noted, but no particular trend
emerged. However, at a level of 5 µg/kg feed, four squamous cell
tumours of the lung, four neoplastic nodules (hyperplastic nodules),
and two cholangiocarcinomas of the liver were found in seven rats.

Kociba et al. (1978) fed groups of 50 male and female Sprague
Dawley rats 0.1, 0.01, and 0.001 µg TCDD/kg body weight for 2 years.
86 male and 86 female control rats received the vehicle only. The
doses corresponded to 2193, 208, and 22 ng TCDD/kg diet. A variety of
tumours were found in the control and experimental groups. Tumours
caused by the ingestion of TCDD were confined to the liver, lungs,
hard palate/nasal turbinates, and tongue. In the female rats that had
received doses of 0.1 and 0.01 µg/kg body weight, a statistically
significant increase of neoplastic nodules (hyperplastic nodules,
hepatomas) of the liver was noted, and in the rats that had received
0.1 mg TCDD/kg body weight there was a statistically significant
increase of hepatocellular carcinomas. Epithelial tumours along the
respiratory tract, tongue, and hard palate consisted of well
differentiated squamous cell carcinomas. There was an increased
incidence, compared with the controls, of squamous cell carcinomas of
the hard palate and nasal turbinate in both male and female rats
receiving 0.1 µg TCDD/kg body weight, while the incidence of squamous
cell carcinoma of the lungs at this dose showed an increase only in
the females. The authors also noted a decreased incidence of tumours
of the pituitary gland, uterus, mammary glands, pancreas, and adrenal
glands in the treated groups, possibly secondary to an effect on the
hormonal functions of different glands. This decrease was in some
instances statistically significant.

Two further studies on the carcinogenicity of TCDD are available
(NIH, 1982a; NIH, 1982b). The TCDD used in these studies was reported
to be 99.4% pure, based on gas chromatographic analysis.

In two gavage studies both Osborne-Mendel rats and B6C3F1 mice
were used (NIH, 1982a). All animals were about 6 weeks old. Dosages,
duration, and outcome are summarized in Table 59. The statistical
analysis performed was similar to that in the dermal study (NIH,
1982b). Mean body weights of the high-dose groups of rats were lower
than those of the corresponding controls after week 55 and 45 for
males and females, respectively, but no other clinical signs were
observed. No such dose-related depression in mean body weight gain was
observed in mice when compared to the vehicle-control groups.

Table 59. Carcinogenicity bioassays of PCDDs after oral administration

```
_____

    Compound                     Exposure: route, dose, frequency    Species/
strain/sex       Tumour type and incidence
    (Reference)                  and duration-treatment/test

_____

    2,3,7,8-TCDD                 Oral (diet), 0.0, 0.001, 0.005,     Rat/Sprague
Dawley/M     all tumours: 0/10 at 0.0,
    (Van Miller et al.,          0.05, 0.5, 1.0, 5.0
µg/kg.                                    0/10 at 0.001, 5/10 at 0.005,
      1977)
78/95 weeks                                               3/10 at 0.05, 4/10
at 0.5, 4/10
                                                                              at

1.0, and 7/10 at 5.0 µg/kg


    2,3,7,8-TCDD                 Oral (diet), 0.0, 0.001, 0.01,      Rat/Sprague
Dawley/M     squamous cell carcinoma hard
    (Kociba et al., 1978)    0.1 µg/kg body weight
per day.                             palate: 4/50 at 0.1 µg/kg per day;
                             105/105
weeks                                      squamous cell carcinoma tongue:
                                                                          1/50
at 0.001 and 0.01, 3/50 at

                                                                          0.1
µg/kg per day; adenoma of

                                                                          adrenal
cortex: 2/5 at 0.01 and

                                                                          5/50
at 0.1 mg/kg per day


                                                          Rat/Sprague
Dawley/F     hepatocellular carcinoma: 0/86

                                                                          at
0.0, 0/50 at 0.001, 2/50 at

                                                                          0.01,
and 11/49 at 0.1 µg/kg per

                                                                          day;
squamous cell carcinoma of

                                                                          tongue:
1/50 at 0.01 and 4/49 at
```

µg/kg per day, squamous cell

of lung: 7/49 at 0.1

kg per day.

0.1

carcinoma

µg/

2,3,7,8-TCDD   Oral (gavage corn oil:acetone,   Rats/Osborne-
Mendel/M   follicular cell adenomas or
(NIH, 1982a)   9:1), 0.0, 0.1,
0.05, 0.5   carcinoma of thyroid: 1/69 at
µg/kg body weight
per week.   0.0, 5/48 at 0.10, 8/50 at 0.05,
104/105-
107 weeks   and 11/50 at 0.5 µg/kg per week

Table 59 (contd - 2).

Compound   Exposure: route, dose, frequency   Species/
strain/sex   Tumour type and incidence
(Reference)   and duration-treatment/test

Rats/Osborne-
Mendel/F   follicular cell adenomas or carcinoma

of

thyroid: 3/73 at 0.0, 2/45 at 0.1,

1/49

at 0.05, and 6/47 at 0.5 µg/kg

per

week; neoplastic nodules or

hepatocellular

carcinoma: 5/75 at

0.0,

1/49 at 0.1, 3/50 at 0.05, and

14/49

at 0.5 µg/kg per week

2,3,7,8-TCDD   Oral (gavage corn oil:acetone,   Mice/
B6C3F$_1$8/M   hepatocellular carcinoma: 8/73
(NIH, 1982a)   9:1), <u>Males</u> 0.0,
0.01, 0.05,   at 0.0, 9/49 at 0.01, 8/49 at
0.5 µg/kg body

Compound	Exposure: route, dose, frequency	Species/strain/sex	Tumour type and incidence
	weight per week. Females 0.0, 0.04, 0.2, 2.0 µg/kg body weight per week, 104/105 weeks	Mice/ B6C3F$_1$8/F	0.05, and 17/50 at 0.5 µg/kg per week hepatocellular carcinoma: 1/73 at 0.0, 2/50 at 0.04, 2/48 at 0.2, and 6/47 at 2.0 µg/kg per week; follicular cell adenomas of thyroid: 0/69 at 0.0, 3/50 at 0.04, 1/47 at 0.2, and 5/46 at 2.0 µg/kg per week
1,2,3,6,7,8/ 1,2,3,7,8,9- hexaCDD (1:2) (NIH, 1980b)	Oral (gavage corn oil:acetone, 9:1), 0.0, 1.25, 2.5, 5.0 µg/kg body weight per week, 104/105 weeks µg/kg per	Rats/Osborne- Mendel/M	liver neoplastic nodules or hepatocellular carcinoma: 0/74 at 0.0, 0/49 at 1.25, 1/50 at 2.5, and 4/48 at 5.0 µg/kg per week
		Rats/Osborne- Mendel/F	liver neoplastic nodules or hepatocellular carcinoma: 5/75 at 0.0, 10/50 at 1.25, 12/50 at 2.5, and 30/50 at 5.0 µg/kg per week

Table 59 (contd - 3).

Compound	Exposure: route, dose, frequency	Species/strain/sex	Tumour type and incidence

```
     (Reference)                  and duration-treatment/test
```

```
     1,2,3,6,7,8-/             Oral (gavage corn oil:acetone,      Mice
(B6C3F1)/M           hepatocellular adenomas or carcinomas:
     1,2,3,7,8,9-                9:1), Males 0.0,
1.25, 2.5,                                   15/73 at 0.0, 14/50 at 1.25, 14.49 at
     hexaCDD (1.2)            5.0 µg/kg body weight
per week                                 2.5, and 24/48 at 5.0 µg/kg per
     (NIH, 1980b)             Females 0.0, 2.5, 5.0,
10.0 µg/                            week
                             kg body weight per week,
                             104/105-108 weeks                      Mice
(B6C3F1)/F           hepatocellular adenomas or carcinomas:
```

```
                                                                    3/73
at 0.0, 4/48 at 2.5, 6.47 at 5.0,
                                                                    and
10/47 at 10.0 µg/kg per week
```

```
     Dibenzo-p-dioxin          Oral (diet), 0, 5000, 10 000        Mice/
B6C3F1/M           hepatocellular carcinoma: 4/49 at 0,
     (NCI, 1977)               µg/kg diet, 87-90/91-
97 weeks                                 7/50 at 5000, and 3/48 at 10 000
```

```
                                                                    µg/
kg diet; hepatocellular adenomas:
                                                                    4/49
at 0, 1/50 at 5000, and 2/48 at
                                                                    10,000
mg/kg diet; malignant
                                                                    tumours:
5/49 at 0, 11/50 at 5000,
                                                                    and
8/50 at 10 000 mg/kg diet
```

```
                                                            Mice/
B6C3F1/F           malignant tumours: 8/50 at 0,
                                                                    9/49
at 5000, and 3/39 at 10,000
                                                                    mg/
kg diet; hepatocellular
                                                                    carcinoma:
1/47 at 5000 mg/kg
                                                                    diet
```

```
     2,7-diCDD                 Oral (diet), 0, 5000, 10 000        Rats/Osborne-
```

```
Mendel/M      malignant tumours: 5/33 at 0,
    (NCI, 1979)                 mg/kg diet, 110/110-
117 weeks.                              7/34 at 5000, and 4/33 at 10 000
                                                                              mg/

kg diet; hepatocellular
                                                                              adenoma:

1/33 at 0; hepatocellular
                                                                              carcinoma:

1/33 at 10,000 mg/kg diet


                                                  Rats/Osborne-
Mendel/F      malignant tumours: 5/31 at 0;
                                                                              4/33

at 5000, and 5/30 at
                                                                              10

000 mg/kg diet
```

In the male rats, increased incidences of follicular cell
adenomas or carcinomas in the thyroid were dose related and were
significantly higher (P < 0.001) in the high-dose group than in the
vehicle controls (1%, 10%, 16%, and 22%). In the female rats, an
increase (though not statistically significant) was seen only in the
high-dose group (4%, 4%, 2%, and 13%). The incidence of neoplastic
nodules of the liver in the high-dose group of female rats was
significantly (P < 0.006) higher than that in the vehicle-control
group (7%, 2%, 6%, and 28%).

In male and female mice, incidences of hepatocellular carcinomas
were dose related and, in the high-dose groups, were significantly (P
< 0.002 and 0.014, respectively) higher than those in the
corresponding vehicle-control groups (males: 11%, 18%, 16%, and 34%;
females: 1%, 4%, 4%, and 13%).

Follicular cell adenomas in the thyroid occurred at dose-related
incidences in female mice, and were significantly (P < 0.009) higher
in the high-dose groups than those in the vehicle controls (0%, 6%,
2%, and 11%). In conclusion, under the conditions of this bioassay,
TCDD was carcinogenic for Osborne-Mendel rats, inducing follicular
cell thyroid adenomas in males and neoplastic nodules of the liver in
females. TCDD was also carcinogenic for B6C3F1 mice, inducing
hepatocellular carcinomas in males and females and follicular cell
thyroid adenomas in females.

Toth et al. (1979) administered TCDD orally by gavage to groups of 45 male Swiss/H/Riop mice at doses of 0, 0.007, 0.7, and 7 µg/kg body weight once a week for one year, and the animals were followed for their lifetime. Liver tumours were found at 18%, 29%, 48%, and 30%, respectively. The tumour incidence at 0.7 µg/kg was significantly higher when compared to controls (P < 0.01), while the increase at the highest dose level (7 µg/kg) was not statistically significant (P = 0.11). The latter finding may be due to a much reduced average survival in comparison with the control group (average life span 424 and 588 days, respectively).

In a study using dermal application of TCDD (NIH, 1982b), male and female Swiss-Webster mice were about 6 weeks old at the beginning of the bioassay. The one-tailed Fisher exact test was used to compare the tumour incidence of a control group with that of a group of dosed animals. Mean body weights of dosed animals were essentially the same as those of the corresponding vehicle-control groups, but less than those of the untreated controls, for males throughout the study and for females during the first 80 weeks. The incidence of fibrosarcoma in the integumentary system of female mice treated with TCDD or TCDD and dimethylbenzathraline (DMBA) was significantly higher than that of the controls (P < 0.007 and P < 0.010, respectively). An increase in the same tumour type, although not statistically significant (P = 0.084), was also observed in the male mice (7% and 21% for the control

and TCDD-treated groups, respectively). In conclusion, under the conditions of this bioassay, TCDD was carcinogenic for female Swiss-Webster mice, causing fibrosarcomas in the integumentary system. However, the study has been criticized in several areas, namely, a maximal tolerated dose (MTD) was not achieved, especially in male mice, only one dose per sex was used, and the number of mice (30) in the TCDD-exposed groups was considered less than optimal.

7.7.2 Long-term animal studies with mixed compounds

Toth et al. (1979) studied groups of 100 male and 100 female, 10-week-old random-bred Swiss H/Riop mice that were given weekly oral doses of 2,4,5-trichlorophenoxyethanol (TCPE) at 67-70 mg/kg body weight, together with 0.112 mg TCDD/kg body weight or 0.007 mg TCDD/kg body weight in 0.5% carboxymethyl cellulose by gastric intubation for 12 months. The incidences of liver tumours in males after 2 years were reported to be 48% and 58% in the two treated groups, compared with 26-33% in the untreated male mice of the colony that survived up to 3 years. Three additional groups of mice were given 7 µg TCPE/kg body weight with 0.0007 µg TCDD/kg body weight, 0.7 µg TCPE/kg body weight

with 0.00007 µg TCDD/kg body weight, or 7 µg TCPE/kg body weight with
0.7 µg TCDD/kg body weight. There was no increased incidence of liver
tumours in any of the treatment groups.

A 1:2 mixture of 1,2,3,6,7,8- and
1,2,3,7,8,9-hexa-chlorodibenzo-p-dioxins (HxCDDs) has been tested for
carcinogenicity by dermal application to mice and by gavage in rats
and mice (NIH, 1980a,). The following impurities were detected in the
mixture: pentaCDD 0.04%, TCDD 0.09%, triCDD 0.004%, and bromopentaCDD
< 0.004%. The specific isomers of these impurities were not
identified. The doses used and duration of the gavage studies (NIH,
1980b) are given in Table 59. In both species and either sex, only
tumours of the liver occurred at a significantly greater incidence
than controls. In male rats and male and female mice, the liver tumour
incidence was significantly increased over control values only in the
high dose groups (5 µg/kg per week), while in female rats the
incidence was significantly greater at both medium- and high-dose
levels (2.5-5 µg/kg per week). In the dermal study, no
treatment-related tumours were recorded in either the carcinogenicity
bioassay or the tumour promotion assay using DMBA as an initiator
(NIH, 1980a). It was concluded that the mixture of hexaCDDs tested was
carcinogenic to rats and mice following administration by gavage.
However, there was no tumorigenic activity when hexaCDD was applied to
mouse skin.

When added to the diet in concentrations up to 10 000 µg/kg,
2,7-dichlorodibenzo-p-dioxin and dibenzo-p-dioxin were found to be
non-carcinogenic in chronic feeding studies in mice and rats of either
sex (NCI, 1977; NCI, 1979).

7.7.3 Short-term and interaction studies

Poland & Glover (1979) estimated the maximum covalent binding of
TCDD in vivo to rat liver protein, ribosomal RNA (rRNA), and DNA
after 3H-TCDD (39 Ci/mmol) was administered to immature male and
female Sprague Dawley rats (105-135 g) as a single ip injection of 7.5
µg/kg. The rats were killed 12 h, 24 h, 48 h, or 7 days after dosing
with TCDD. The level of radioactivity in the liver varied from 18 to
64% of the administered dose, and only a small fraction was associated
with the purified macromolecular fractions. The radioactivity
associated with rRNA and DNA was very low and essentially all the
unextracted radioactivity was associated with protein (0.03 to 0.1% of
the total radioactivity in the liver). The maximum amount of ^{3}H-TCDD
that could have been covalently bound to DNA was estimated as 1.8 x
10^{-17} mol TCDD per mg DNA, or 6.2 nmol TCDD per mol DNA nucleotide,
which means binding of about 1 molecule TCDD to the DNA in 35 cells.

Phenobarbital treatment, or prior administration of TCDD did not significantly alter the amount of unextractable ^{3}H-TCDD associated with any macromolecular fraction. Similarly, there were no differences in the levels of ^{3}H-TCDD associated with protein, rRNA, or DNA in male or female rats pretreated with TCDD.

TCDD was found to be a carcinogen in chronic feeding studies in rats and mice. Most carcinogens bind covalently, either directly or after a conversion to electrophilic intermediates, to protein, rRNA, and DNA to the extent of 10^{-4} to 10^{-6} mol of carcinogen per mol of amino acid or nucleotide residue. The maximum binding of TCDD is 4-6 orders of magnitude lower than that of most chemical carcinogens and is of questionable biological significance. The results obtained in the study of Poland & Glover (1979) thus indicate that it is unlikely that the mechanism of TCDD-induced carcinogenesis would include the covalent binding of TCDD.

In female Charles River CD-1 mice, TCDD was found to be a weak initiator when given alone in a single dose of 2 µg/ mouse by dermal application (Di Giovanni et al., 1977). In these studies 12-O-tetradecanoylphorbol-13-acetate (TPA) was used as a promoter. When TCDD and 7,12-dimethylbenzanthracene were given together, a slight additive effect was found. As mentioned earlier, in a study on a hexaCDD mixture, no treatment-related tumours were found in a tumour promotion test on mice using DMBA as an initiator (NIH, 1980a).

The possible role of TCDD as a promoter in diethylnitrosamine-induced hepatocarcinogenesis was studied by Pitot et al. (1980) in female Charles River rats (200-250 g). A single oral dose (10 mg/kg) of diethylnitrosamine (DEN) was given 24 h after a 70% hepatectomy, and treatment with TCDD (0.14 or 1.4 mg/kg sc once every 2 weeks for 7 months) was started one week after the hepatectomy. The promoting effect of TCDD in this 2-stage model of liver cancer was also compared with the effect of a known promoting agent, phenobarbital (0.05% in the diet for 7 months). Enzyme-altered

foci, which are thought to be precursors of hepatocellular carcinomas, were greatly increased in number, total volume, and phenotypic heterogeneity by the administration of TCDD. A significant incidence of hepatocellular carcinomas (5 out of 7) was observed in the DEN-treated rats that were given the high dose of TCDD, but no carcinomas were seen in the rats treated with DEN only (0 out of 4). The results indicated that TCDD was a potent promoting agent for hepatocarcinogenesis, and the authors suggested that all the tumours associated with the chronic administration of TCDD arise from its promoting activity of cells previously initiated by exposure to

carcinogens in the environment.

Studies utilizing a two-stage system of mouse skin tumorigenesis (Berry et al., 1979), which allows separate evaluation of the initiation and promotion phases of carcinogenesis, have demonstrated that TCDD does not promote the development of skin tumours at a dose of 0.1 µg given twice weekly, whereas in the animals pretreated with 1.0 µg TCDD for 1, 3, or 5 days prior to initiation with DMBA, TCDD was shown to act as a potent inhibitor of PAH-induced skin tumour initiation. Almost complete inhibition (96%) was achieved with a single non-toxic topical dose of 0.1 µg, and 3 days pretreatment with 0.01 µg TCDD gave over 80% inhibition. The authors suggested that this potent anticarcinogenic effect of TCDD may be related to its ability to induce epidermal enzyme pathways involved in detoxifying PAH carcinogens in the skin. According to Kimbrough (1979), TCDD and other compounds of this type, which are potent enzyme inducers, may prevent or enhance the tumour-inducing ability of other chemicals by enhancing the metabolism of these xenobiotics.

Poland et al. (1982) studied the promoting effects of TCDD in the mouse skin two-stage tumorigenesis model. The effects of TCDD and TPA were compared in DMBA-initiated HRS/S mice that were either heterozygous or homozygous for the recessive "hairless" trait. TCDD was found to have a tumour-promoting effect only in the homozygous mice. The data suggested to the authors that TCDD might act as a promoter by a mechanism different from that of TPA.

The interaction of TCDD with 3-methylcholanthrene (3-MC) was studied by Kouri et al. (1978), who found that TCDD was a co-carcinogen with 3-MC when administered by subcutaneous injection. Both sexes of two inbred strains of mice (C57Bl/6C and DBA/2), responsive and non-responsive to the induction of AHH by 3-MC, respectively, were used. TCDD at a concentration of 1 or 100 µg/kg body weight was administered as a single dose alone or in combination with 3-MC (150 µg/kg). The duration of the study was 36 weeks. The number of animals in each group at the start of the experiment was not stated, but seems to have been between 30 and 100. No subcutaneous tumours were observed in controls or in mice treated with TCDD alone.

In responsive mice no enhancement occurred, while in non-responsive mice the simultaneous administration of TCDD and 3-MC enhanced the carcinogenic response of TCDD at 100 mg/kg. At 1 mg TCDD/kg, a reduction in latency time to tumour was noted.

An anticarcinogenic effect of TCDD has been reported by Cohen et al. (1979) and by Di Giovanni et al. (1979a). When TCDD was topically

applied to Sencar or CD-1 mice 72 h prior to the administration of
either DMBA (10 nmol) or benzo(a)-pyrene (BP) (100 nmol), it markedly
decreased the skin tumour initiation by both DMBA and BP. This
inhibition of tumorigenesis correlated with the decreased in vivo
binding of DMBA to DNA after TCDD administration, but not with the
total binding of BP to DNA. However, the
hydrocarbon-deoxyribonucleoside adducts from the DNA of
TCDD-pretreated mice showed a striking absence of
BP-7,8-dihydrodiol-9,10-epoxide adduct bound to guanine. It is
suggested, accordingly, that the formation of this adduct may be a
critical step in BP-induced skin carcinogenesis in mice. In further
studies of the tumour-inhibitory effect of TCDD (Di Giovanni et al.
1980), it was demonstrated that exposure of CD-1 mice to TCDD 3 days
before initiation with BP or 3-MC resulted in a decreased tumour
yield, compared to acetone-pretreated animals, while treatment with
TCDD 5 min before and 1 day after initiation failed to affect the
tumour yield. However, when TCDD was administered 3 days or 5 min
before or 1 day after initiation with BP-diol epoxide, there was a
decreased tumour yield in all cases. The authors concluded that the
ability of TCDD to inhibit tumour yield when administered after the
BP-diol epoxide, indicated the possible existence of more than one
mechanism involved in the anticarcinogenic effect of TCDD.

7.8 Mechanisms of Action

     The toxicity of TCDD apparently depends on the fact that the four
lateral positions of the molecule are occupied by chlorine (see
section 7.8.1) Toxicity decreases with decreasing lateral substitution
and increasing total chlorine substitution. As has been outlined in
sections 7.1-7.7, TCDD toxicity involves many different types of
symptoms and these symptoms vary from species to species and from
tissue to tissue, both quantitatively and qualitatively. Furthermore,
age- and sex-related differences in sensitivity to TCDD have been
reported. Characteristic for TCDD toxicity is also the delay in
expression of toxicity, from 2 weeks to 2 months, seen in all species.
It has been suggested that the initial event in TCDD-induced toxicity
is the binding of TCDD to the so-called Ah receptor. This complex,
whether of cytosolic or nuclear origin, exerts its action in the
nucleus by triggering a pleiotropic response including the induction
of mixed function oxidases. Present knowledge, however, rules out
enzyme induction per se as being the cause of toxicity and death
(see section 7.8.1). Although the toxicokinetics of TCDD vary between
species, these differences are not sufficient to explain the
variabilities in sensitivity to TCDD toxicity (see section 7.8.2).

Available data indicate an involvement of TCDD in processes regulating

cellular differentiation and/or division. Alterations in the
regulation of such processes, which are not equally active in all
cells throughout the organism, would be expected to result in effects
that vary among tissues as well as among species (see section 7.8.3).

7.8.1 Receptor-mediated effects

     The binding of TCDD to the Ah receptor has been postulated to be
the necessary first step in the induction of cytochrome P-450
synthesis and of related enzyme activities, as well as in the
mechanism of toxicity (Poland et al., 1976; Okey et al., 1979; Poland
& Glover, 1979; Poland & Knutson, 1982). So far, no conclusive data
exist for the direct involvement of the Ah receptor in the
TCDD-induced toxicity.

     Knowledge of the mechanism involved in the Ah locus
enzyme-induction response has grown rapidly since the initial indings
that binding of TCDD to the receptor resulted in increased levels of
cytochrome P-450 mRNA in genetic variants of mice (Tukey et al., 1982)
and mouse hepatoma cell lines (Israel & Whitlock, 1983). These
findings have been confirmed and further expanded in studies using
mice genetics and recombinant DNA techniques (Tukey et al., 1982;
Miller et al., 1983; Gonzales et al., 1984; Israel & Whitlock, 1984;
Jones et al., 1984, 1985, 1986; Okino et al., 1985; Tuteja et al.,
1985; Kimura et al., 1986), thus providing more data to the
understanding of the mechanism for the Ah locus enzyme induction.

     In early experiments (Poland et al., 1976; Carlstedt-Duke, 1979;
Okey et al., 1979), Ah receptors appeared to be localized in the
cytosol when in its unoccupied state and was translocated into nuclei
only when occupied by a ligand (Greenlee & Poland, 1979; Okey et al,
1980; Poellinger et al., 1982; Gasiewicz & Rucci, 1984). However,
Whitlock & Galeazzi (1984) concluded that unoccupied Ah receptor in
the intact cell was primarily located in the nucleus and that apparent
cytosolic Ah receptor was a redistribution artifact. Following the
distribution of Ah receptor and three cytosolic marker enzymes between
the nuclear and cytosolic fractions during fractionation (Denison et
al., 1986a,c), it was again concluded that unoccupied Ah receptor is
primarily cytosolic or that this receptor protein is in equilibrium
between the cytoplasm and nucleus.

     However, it is generally agreed that the ultimate biological
regulation by the Ah receptor is due to specific interaction of
ligand-receptor complexes with chromatin sites (Greenlee & Poland,
1979; Okey et al., 1979, 1980; Mason and Okey, 1982; Poellinger et
al., 1982; Poland and Knutson, 1982; Tukey et al., 1982a; Gonzales et

al., 1984; Israel & Whitlock, 1984).

Table 60. Physicochemical data for the hepatic Ah receptor in Sprague
Dawley rats

Physicochemical data	Denison et al. (1986a)	Poellinger et al. (1983)
Stokes radius (nm)	5.2 ± 0.2	6.1 ± 0.2
Sedimentation coefficient (S)	5.6 ± 0.6	4.4
Relative molecular mass	121 000	111 000

Several investigators have estimated the molecular size and other
physicochemical properties for the cytosolic hepatic Ah receptor (Okey
et al., 1979, 1980, 1982; Tukey et al., 1982b; Poellinger et al.,
1983; Gasiewicz et al., 1983a,b; Denison et al., 1986a; Hannah et al.,
1986). To obtain reliable results in the isolation and
characterization of the Ah receptor, it is necessary to use perfused
liver, in order to reduce the contribution from blood proteins, and to
use a radioactive ligand of high purity and specific activity quality
(Poellinger et al., 1983; Denison et al., 1986a). Further more, the
ionic strength of the medium during isolation has a marked effect upon
the apparent molecular weight of the receptor (Denison et al., 1986a).
The physicochemical data for the Ah receptor presented in Table 60
were obtained from two studies (Denison et al., 1986a,c; Poellinger et
al., 1983) in which the receptor was isolated from perfused liver of
Sprague Dawley rats under conditions of high ionic strength.

The receptor protein has been found also in extrahepatic tissues
(Carlstedt-Duke, 1979; Carlstedt-Duke et al., 1979, 1981; Johansson et
al., 1982; Mason & Okey, 1982; Gasiewicz & Rucci, 1984; Gasiewicz et
al., 1984; Furuhashi et al., 1986; Kurl et al., 1985; Söderkvist et
al., 1986). Different mammalian species possess Ah receptors with
similar, though not identical, properties (Gasiewicz & Rucci, 1984;
Denison et al., 1986a,b; Kurl et al., 1985). Jaiswal et al. (1985a,b)
have shown species differences in the TCDD-inducible P-450 gene
subfamily. Humans appear to only have the P1-450. The function of
human P1-450 may be equivalent to a combination of P1-450 and P3-450
in the mouse. A complete lack of measurable cytosolic and almost
total absence of inducer-receptor complexes in the nucleus of human

MCF-1 cells (cells derived from an adenocarcinoma of the breast) were reported. This absence was out of proportion to the ability of TCDD to induce AHH and acetamide-4-hydroxylase activities in these cells. Further studies in different cell lines are thus needed to characterize the level of receptor in humans. The only non-mammalian

species demonstrated to have significant Ah receptor is the chick embryo (Denison et al., 1986b). However, no detectable level of the receptor was found in 2-week-old White Leghorn chickens (Sawyer et al., 1986). Based on certain similarities in the biochemical behaviour between the Ah receptor and steroid hormone receptors, it has been proposed that there is a natural ligand for the Ah receptor (Neal et al., 1979; Poland et al., 1976). So far such a ligand has not been identified, either among steroid hormones (Poland et al., 1976; Carlstedt-Duke et al., 1979; Romkes et al., 1987) or among certain dietary factors (Johansson et al., 1982), although lumichrome, a metabolite of riboflavin, was suggested as an endogenous ligand for the receptor (Kurl & Villee, 1985). TCDD does not bind to the glucocorticoid, estrogen or progesterone receptors (Neal et al., 1979; Romkes et al., 1987). Monoclonal anti-glucocorticoid receptor-IgG antibodies did not react with the TCDD receptor (Poellinger et al., 1983) and the hydrophobic properties of the Ah receptor were more pronounced than those of the steroid hormone receptors (Poellinger & Gullberg, 1985).

Convincing data for the importance of the receptor in TCDD-induced toxicity could be based on structure activity relationships, i.e., that the binding affinities of TCDD and other PCDDs or PCDFs to the receptor correlate with their biological potencies. The binding affinities of PCDDs and PCDFs have been demonstrated to correlate with their biological potencies, particularly the induction of enzyme activities as well as the production of acute toxic effects (Tables 56 and 61) (Poland & Kende, 1976; Poland et al., 1976; Knutson & Poland, 1982).

Furthermore, the structure-activity relationships observed for enzyme induction, thymic atrophy, body weight loss, and LD50 values were comparable to the structure-activity relationships observed for receptor binding (Tables 56, 61) (Bandiera et al., 1984a,b; Mason et al., 1985, 1986; Sawyer & Safe, 1985; Safe et al., 1986). Interactive studies, i.e., studies where PCDD and PCDF congeners have been given both separately and as mixtures, have also been used to investigate the role of the Ah receptor in the mechanism of action of TCDD. Depending on the mechanism of action the biological responses may be synergistic potentiated, additive, unaffected, or antagonistic. Such studies have been performed for enzyme induction (Sawyer & Safe, 1985;

Keys et al., 1986; Ahlborg et al., 1987), vitamin A reduction (Hakansson et al., 1987), teratogenicity (Birnbaum et al., 1985; Weber et al., 1985), thymic atrophy (Bannister & Safe, 1987), and immune suppression (Rizzardini et al., 1983). So far this kind of data is scattered and difficult to interpret.

Table 61. Structure-activity relationships for some PCDFs

PCDF congener	In vitro EC_{50} values (mol/litre)[a,b]			In vivo ED_{50} values (µmol/kg)		LD_{50} [c]
	Receptor binding	AHH EROD	AHH	weight loss	Thymic atrophy	Guinea-pig µg/kg body weight
Dibenzofuran	$< 10^{-3}$	ND	ND			
2-	2.8×10^{-4}	ND	ND			
3-	4.2×10^{-5}	ND	ND			
4-	$< 10^{-3}$	1.0×10^{-5}	1.71×10^{-5}			
2,3-	4.72×10^{-6}	2.19×10^{-6}	4.84×10^{-6}			
2,6-	2.46×10^{-4}	6.17×10^{-5}	6.31×10^{-5}			
2,8-	2.57×10^{-4}	3.95×10^{-5}	4.0×10^{-5}			
1,3,6-	4.40×10^{-6}	2.53×10^{-6}	3.37×10^{-6}			
1,3,8-	8.50×10^{-5}	1.94×10^{-5}	3.02×10^{-5}			
2,3,4-	1.9×10^{-5}	1.51×10^{-7}	2.48×10^{-7}			
2,3,8-	1.0×10^{-6}	2.49×10^{-6}	1.56×10^{-6}			
2,6,7-	4.5×10^{-7}	2.80×10^{-6}	3.13×10^{-6}			
2,3,4,6-	3.5×10^{-7}	1.32×10^{-6}	1.13×10^{-6}			
2,3,4,7-	2.51×10^{-8}	1.79×10^{-8}	1.48×10^{-8}	46	34	7.8
2,3,4,8-	2.0×10^{-7}	4.14×10^{-8}	3.76×10^{-8}	ND	130	> 150
2,3,6,8-	2.2×10^{-7}	1.04×10^{-6}	7.79×10^{-7}			
2,3,7,8-	4.1×10^{-8}	3.91×10^{-9}	2.02×10^{-9}			

```
   0.65              3.2          3.6                 5-10
      1,2,3,6-          3.54 x 10⁻⁷           > 10⁻⁴               > 10⁻⁴            >
160            > 250        > 250
      1,2,3,7-          1.12 x 10⁻⁷          2.7  x 10⁻⁵          6.3  x 10⁻
⁵     110                  87         110
      1,2,4,8-          > 10⁻⁵                1.20 x 10⁻⁵          9.26 x 10⁻⁵
      1,2,4,6,7-        6.77 x 10⁻⁸          3.25 x 10⁻⁷          3.48 x 10⁻⁷
      1,2,4,7,9-        2.0  x 10⁻⁵          3.77 x 10⁻⁸          3.84 x 10⁻⁸
      1,2,3,4,8-        1.2  x 10⁻⁷          2.09 x 10⁻⁷          1.63 x 10⁻⁷
      1,2,3,7,8-        7.45 x 10⁻⁸          2.54 x 10⁻⁹          3.06 x 10⁻⁹
1.5              2.6          1.8
      1,2,3,7,9-        3.98 x 10⁻⁷          8.6  x 10⁻⁸          8.6  x 10⁻
⁸       15                  49         23
      1,2,4,6,8-        3.09 x 10⁻⁶           1.0  x 10⁻⁵          1.2  x 10⁻⁵     >
150             7150        > 150
```

Table 61 (contd).

In vitro EC_{50} values (mol/litre)[a,b]   In vivo

ED_{50} values (µmol/kg) LD_{50} [c]

```
      PCDF            Receptor            AHH
EROD              AHH              weight     Thymic    Guinea-pig
      congener
binding
loss       atrophy   mg/kg body weight
```

```
      1,2,4,7,8-    1.3  x 10⁻⁶        1.06 x 10⁻⁷        1.48 x 10⁻
⁷       7.8                 49         46
      1,3,4,7,8-    2.0  x 10⁻⁷        1.60 x 10⁻⁹        1.40 x 10⁻
⁹       3.5                 26         0.70
      2,3,4,7,8-    1.5  x 10⁻⁸        2.56 x 10⁻¹⁰       1.34 x 10⁻
¹⁰      0.037               1.0        0.26
      2,3,4,7,9-    2.0  x 10⁻⁷        7.9  x 10⁻⁹        5.8  x 10⁻
⁹       7.0                 22         5.5
      1,2,3,4,7,8-  2.3  x 10⁻⁷        3.56 x 10⁻¹⁰       3.79 x 10⁻
¹⁰      0.29                1.3        0.56
      1,2,3,6,7,8-  2.7  x 10⁻⁷        1.47 x 10⁻⁹        1.24 x 10⁻
```

```
9       0.35              3.2          0.90
    1,2,4,6,7,8-   8.3  x 10⁻⁶      4.24 x 10⁻⁸       2.93 x 10⁻⁸
    2,3,4,6,7,8-   4.7  x 10⁻⁸      6.87 x 10⁻¹⁰      5.75 x 10⁻
10      0.27              2.8          0.90
```

9	0.35	3.2	0.90
1,2,4,6,7,8-	8.3×10^{-6}	4.24×10^{-8}	2.93×10^{-8}
2,3,4,6,7,8-	4.7×10^{-8}	6.87×10^{-10}	$5.75 \times 10^{-}$
10	0.27	2.8	0.90

[a]   Estimated concentration needed to displace 50% of ^{3}H-TCDD bound to liver cytosol receptor from Wistar rats and to produce 50% maximum enzyme induction in the rat hepatoma H-4-IIE cell line (Bandiera et al., 1984b).

[b]   Studies in immature male Wistar rats (Mason et al., 1985).

[c]   Moore et al. (1979).

Polymorphism in the Ah locus, which is suggested to be structural gene for the cytosolic receptor, seems to determine the sensitivity of genetically different strains of mice to TCDD and congeners. Ah responsive strains of mice, e.g., C57Bl/6, are characterized by (a) high hepatic levels of the TCDD receptor protein, (b) highly elevated levels of hepatic cytochrome P-448 and associated enzyme activities in response to treatment with 3-MC, and (c) sensitivity to the ulcerative action of DMBA on the skin. Ah-non-responsive mice, e.g., DBA/2, lack these attributes (Nebert et al., 1975). Based on these findings several genetic studies have been performed to elucidate the role of the receptor in TCDD toxicity. Contrary to 3-MC, TCDD induces AHH activity and several toxic effects both in Ah-responsive and Ah-non-responsive strains of mice. However, the dose required to produce the effect in an Ah-non-responsive strain is approximately 10-fold greater than that needed for a responsive strain, thus demonstrating that the Ah-non-responsive strain also contains the TCDD-receptor but that this receptor is defective (Okey & Vella, 1982).

Crosses and backcrosses of C57BL/6 and DBA/2 mice have shown that sensitivity to TCDD-induced thymic atrophy immune system disturbances (section 7.4.5) and teratogenic effects (section 7.5.2) segregate with the Ah locus. Furthermore, data from studies of DBA/2 mice given either single or multiple doses of TCDD (Jones & Sweeney, 1980; Smith et al., 1981) suggest that the LD_{50} in this strain of mice is at least 5-fold greater than the values recorded for the C57Bl/6 and C57Bl/10 strains (Vos et al., 1974; Jones & Greig, 1975; Smith et al., 1981). TCDD-induced hepatic porphyria has also been shown to segregate

with the Ah locus in mice (Jones & Sweeney, 1980). However, Greig et al. (1984) found that additional genetic loci must be involved in this lesion. The correlative differences between the C57Bl/6 and DBA/2 strains of mice, in terms of altered specific binding of TCDD and sensitivity to this compound, may be unique and may not be applicable to other species (Gasiewicz & Rucci, 1984).

Less convincing data for the model of receptor-mediated toxicity of TCDD arise from studies of toxicity, receptor levels, and/or enzyme induction of TCDD in various species, tissues, and cell cultures. Despite enormous variability in recorded LD50 values for guinea-pig, rat, mouse, rabbit, and hamster (Table 47), the amounts and physical properties of the hepatic as well as the extrahepatic receptors, do not vary extensively in these species (Poland & Knutson, 1982; Gasiewicz & Rucci, 1984). Furthermore, although recorded LD_{50} values for TCDD vary more than 100 times in chick embryos, C3H/HeN mice, and Sprague Dawley rats, the ED_{50} doses for AHH induction in these species are comparable (Poland & Glover, 1974b). In the guinea-pig, the most TCDD-susceptible species, enzyme induction is several times lower even at lethal doses. A number of cell types, including primary

cultures and established and transformed cell lines from several species and tissues, are inducible for AHH activity, indicating the presence of the receptor, yet toxicity is not expressed in these systems (Knutson & Poland, 1980a).

Available data thus suggest that the receptor for TCDD may be a prerequisite, but is not sufficient in itself for the expression of TCDD toxicity.

7.8.2 Toxicokinetics

The interspecies variation in sensitivity to TCDD may be attributable, at least in part, to different rates at which various species distribute, metabolize, and excrete the compound. Table 62 summarizes some of the data on the elimination, toxicity, and metabolism of TCDD in different species, some previously discussed in section 6. Distribution data (section 6.1) has been obtained mainly from animals exposed to toxic doses of TCDD. Interspecies comparisons based on these data are difficult to perform, since studies in different species have been performed with non-comparable relative toxic doses and collection of data has occurred at variable time points. However, the available data suggest that tissue levels alone cannot explain the interspecies differences in pathology and acute toxicity of TCDD. For example, the molar concentration of TCDD in the

hamster may be orders of magnitude greater than in the rat and mice,
without development of hepatotoxicity, yet definite liver damage
occurs both in rats and mice.

Based on the findings that the toxicity of TCDD is lower in rats
after the stimulation of hepatic mixed function oxidases (Beatty et
al., 1978), and that the metabolites of TCDD are less toxic than the
parent compound (Weber et al., 1982a; Mason & Safe, 1986) (see section
7.1.5), the metabolism of TCDD has been considered as a detoxification
mechanism.

Following the administration of TCDD, most of it appears to be
eliminated through a first-order process in most species (see section
7.1). In all species investigated TCDD is largely eliminated in the
faeces (Table 48). Only in hamsters and certain strains of mice is
urinary elimination a major route of excretion (Table 41). Available
data demonstrate that TCDD is converted to more polar metabolites
prior to elimination in the urine and bile (Table 62). Unchanged TCDD
does not appear in the bile or urine of any species, but it is the
major excretory product in the faeces of mice, guinea-pigs, and
hamsters (Table 62). As the urine and bile appear to be free of
unmetabolized TCDD, the existence of unchanged TCDD in faeces
indicates that a significant amount of unchanged TCDD may be excreted
into the intestinal lumen by some route other than bile.

Table 62. Rates of elimination, toxicity, and metabolic transformation of
TCDD in different species

Species/strain	Elimination half-life (days)	LD_{50} value (μg/kg body weight)	TCDD-derived radioactivity occurring in:			
			Urine	Bile	Faeces	Tissues
Rats[a,b,c,d]						
Sprague Dawley	17-31	25-60	8 polar	5 polar metabolites	4-unchanged TCDD metabolites; little, if any,	

				unchanged TCDD.

Mice[e],[f],[g]

C57BL/6, DBA/2,	10-24	114-2570	4-7 polar	4-6 polar
B6D2F$_1$ metabolites	3-4 polar metabolites	unchanged TCDD metabolites,	metabolites	metabolites
				unchanged TCDD.

Guinea-pig[h]

Hartley	22-94	0.6-2.5	4 polar	5 polar
metabolites	mainly unchanged	unchanged TCDD, polar metabolites	metabolites	metabolites TCDD

Hamsters[a],[i]

Golden Syrian	12-15	1157-5051	4 polar	5-6 polar
metabolites	metabolites, unchanged	unchanged TCDD	metabolites	metabolites TCDD

Dogs[j]

Beagle	not reported	not reported		metabolites

---

[a] Gasiewicz et al., 1983a.   [b] Poiger & Schlatter, 1979.   [c] Ramsey et al., 1982.   [d] Rose et al., 1976.

[e] Gasiewicz et al., 1983b.   [f] Koshakji et al., 1984.   [g] Vinopal & Cassida, 1973.   [h] Olson, 1986.

[i] Olson et al., 1980a.   [j] Poiger et al., 1982.

The metabolic profiles of TCDD in excreta differs between species, but generally urinary metabolites are more polar than biliary or faecal metabolites. Furthermore, several metabolites, both in urine and bile, are glucuronide conjugates.

The apparent absence of TCDD metabolites in the tissues of all species, except for the guinea-pig (Table 62), suggests that, once

formed, the metabolites of TCDD are readily excreted. Another factor, besides metabolism, that may influence the total rate of elimination of TCDD is the amount of adipose tissue stores, which may vary between species.

At present there is no clear relationship between the ability of a given species to excrete TCDD and/or its metabolites and the acute toxicity of TCDD in that species. However, the somewhat greater rate of elimination of TCDD in the hamster and the lower rate of elimination in the guinea-pig (Table 62) may contribute to their relative resistance and sensitivity, respectively, to the acute toxic effects of TCDD.

The rate of metabolism and excretion of PCDDs and PCDFs varies with molecular structure. In most species PCDFs are much more readily eliminated than their PCDD counterparts. Less halogenated congeners are usually metabolised and excreted more rapidly than the more halogenated ones, especially the 2,3,7,8-substituted congeners (see sections 6 and 9).

7.8.3 Impairment of normal cellular regulatory systems

When considered together, the diverse pattern of toxic effects, the species and tissue-specific responses, and the time-course for effects, as well as the non-toxic action of TCDD on most cell cultures in vitro, seem to indicate that TCDD-induced toxicity occurs as a result of an impairment of a normal cellular regulatory system. Such a system might be present in all cells throughout the organism, though the activity may vary with cell type, tissue, age, sex, and strain, and/or species.

7.8.3.1 Endocrine imbalance

In many aspects, TCDD toxicity mimics endocrine imbalance, although no evidence exists to indicate a direct involvement of hormones in the toxic action of TCDD (section 7.4.9).

7.8.3.2 Body weight regulation

The most reliable and consistent symptom of TCDD toxicity among all experimental animals is weight loss. The cause of the body weight loss seems to be reduced food intake, apparently occurring secondarily to a physiological adjustment that reduces the body weight to a

maintenance level lower than normal. The physiological trigger for controlling this body weight set-point might be a target for TCDD

action (section 7.4.1).

7.8.3.3 Plasma membrane function

     The changes in the surface characteristics of the plasma
membranes induced by TCDD in vivo (7.4.2.2) resemble changes
occurring in precancerous and transformed cells (Pitot & Sirica,
1980). Such changes, including reduction of gap junctions and surface
glyco-proteins, would be expected to curtail cell-cell communication
and to reduce intercellular recognition and attachment events
implicated in the process of tumour promotion.

     It has been shown by Pitot et al. (1980) that TCDD promotes
diethylnitrosamine (DEN)-induced hepatocarcinoma in rats. In this
study, canalicular ATPase was used as a marker in detecting
enzyme-altered foci, whose number increased when TCDD was given to
DEN-treated partially hepatectomized rats. The foci exhibited
decreased ATPase activity in agreement with previous observations that
TCDD in vivo reduces the ATPase level in canaliculi-rich plasma
membranes.

     TCDD, unlike other well known promoters, requires a prolonged
treatment period in vivo to exert its effect. The lack of effect
of TCDD in vitro would imply that the promoter activity is
mediated through some in vivo process and not by its direct
interaction with plasma membranes.

7.8.3.4 Impaired vitamin A storage

     The histomorphological appearance of chloracne, the most
characteristic and prominent sign of TCDD-induced toxicity in humans,
resembles in some respects effects seen in the skin of patients
suffering from vitamin A deficiency (Kimbrough, 1974). Many of the
effects of TCDD poisoning observed in animal studies, including
failure of normal growth, keratosis, epithelial lesions,
immunosuppression, and reproductive and teratological effects, are
similar to the effects of dietary vitamin A deficiency (Thunberg et
al., 1980). The most intriguing similarities between symptoms due to
vitamin A deficiency and TCDD toxicity concern effects on epithelial
tissues, particularly the process of keratinization. TCDD induces
terminal differentiation of epithelial tissues both in vivo and
in vitro. However, lack of epithelial degeneration (programmed
cell death) of the medial epithelial cells of palatal shelves has been
reported in mice exposed to TCDD in utero (Pratt et al., 1984).

     Vitamin A is essential for normal differentiation. It diminishes

the expression of differentiation in stratified squamous epithelia and accentuates the expression of differentiation in secretory epithelia. Vitamin A deficiency can convert secretory epithelia to squamous epithelia, while excess of the vitamin can convert stratified squamous epithelia to secretory epithelia (Wolf, 1980). With the use of cultured human keratinocytes, it has been demonstrated that vitamin A at the cellular level affects cell motility, cell-cell interaction, and epithelial morphogenesis. At the molecular level, vitamin A determines, by controlling the level of the corresponding mRNA, the nature of keratins synthesized (Fuchs & Green, 1981). Keratins constitute a cytoskeleton in epithelial cells, and the keratin pattern may be used as a marker for epithelial differentiation (Sun et al., 1979, 1983a,b). Removal of vitamin A from the medium of cultivated human keratinocytes of various origin led to increased synthesis of large keratins and reduced synthesis of lower molecular weight keratins (Fuchs & Green, 1981). This pattern was reversed by the addition of vitamin A to the medium. Each tissue and cell type controlled its synthesis of keratins differently, depending on the vitamin A concentration in the medium. The ability of TCDD to impair vitamin A storage (section 7.4.10) may be responsible for some of the toxic effects produced by TCDD.

7.8.4 Lipid peroxidation

     Based on indirect lines of evidence, Sweeney & Jones (1983) proposed that increased _in vivo_ lipid peroxidation, resulting in the formation of free radicals, might be a mechanism of TCDD toxicity. Firstly, lipofuscin pigments, considered to be by-products of lipid peroxidation, accumulate in the heart muscle cells of TCDD-treated rats (Albro et al., 1978). Secondly, iron deficiency inhibits _in vitro_ lipid peroxidation (Bus & Gibson, 1979; Sweeney et al., 1979) and has been demonstrated to reduce hepatic TCDD toxicity _in vivo_ in rats (Sweeney et al., 1979). Thirdly, 0.25% butylated hydroxyanisole (BHA) in the diet has been shown to provide protection from TCDD-induced porphyria and lipid accumulation in mice. In contrast, 0.01% vitamin E, another antioxidant, in the diet had no protective effect (Hassan et al., 1985a,b). Stohs et al. (1983) demonstrated increased _in vivo_ (conjugated diene method) and _in vitro_ (microsomal malondialdehyde formation) hepatic lipid peroxidation in female Sprague Dawley rats given a total of 70 µg/kg body weight in three daily oral doses of 10, 20, and 40 µg TCDD/kg body weight, or a single oral dose of 80 µg TCDD/kg body weight. Lipid peroxidation was determined at days 1, 6, and 11 after the last treatment. The maximal increase of lipid peroxidation _in vivo_ was 2-fold one day post-treatment, whereas the 5- to 6-fold increase in _in vitro_ lipid peroxidation reached its maximum at 6 days

post-treatment. The TCDD-induced in vitro lipid peroxidation could be inhibited by repeated treatment with BHA, glutathione, vitamin E, and vitamin A (Hassan et al., 1985a,b). Dietary selenium had no inhibitory effect on TCDD-induced lipid peroxidation (Hassan et al., 1985c).

Robertson et al. (1985) found no evidence for TCDD-induced in vivo lipid peroxidation, as judged by levels of exhaled endogenous ethane, and metabolic clearance of both externally and internally applied exogenous ethane in male Sprague Dawley rats after a single ip dose of 60 µg TCDD/kg body weight. Neither was there a correlation between TCDD-induced lipid peroxidation in vitro and sensitivity towards the lethal effect of TCDD in Sprague Dawley rats, Golden Syrian hamsters or guinea-pigs (Hassan et al., 1983). Hepatic microsomes from Sprague Dawley rats and Golden Syrian hamsters exposed to TCDD in vitro responded with increased lipid peroxidation only in the presence of Fe^{3+}-ADP in the incubation mixture (Albro et al., 1986).

From all these data on TCDD and lipid peroxidation, it was concluded by Albro et al. (1986) that it was premature to attempt to define a relationship between lipid peroxidation and TCDD-induced lethality.

8. EFFECT OF PCDDs ON HUMAN BEINGS - EPIDEMIOLOGICAL AND CASE STUDIES

8.1 Occupational Studies - Historical Perspective

The illness most frequently observed in workers engaged in the manufacture of trichlorophenol, 2,4,5-T, and related products is a skin disease called chloracne. This skin disease has also been called "Pernakrankheit" (perchlorinated naphthalene illness or halogen wax acne) and was described by Herxheimer (1899). In addition to the halogenated phenols, chloracne is caused by a number of chlorinated compounds such as the chlorinated biphenyls and chlorinated naphthalenes (Muller, 1937; Braun, 1955; Crow, 1970; Kimbrough, 1974).

Although chloracne is well known to those engaged in the treatment of occupational diseases, many outbreaks that have occurred over the years, particularly in the USA, have not been reported in the scientific literature. In the Federal Republic of Germany, chloracne is now considered an occupational disease for which compensation is mandatory (Braun, 1970).

Herxheimer (1899) also described general toxic signs and symptoms in his patients, such as lack of appetite, weight loss, headache, and vertigo. After his original observations and publication, several other reports followed. The technique of obtaining chlorine gas consisted of an electrolytic procedure where a mixture of potassium, sodium, and magnesium chlorides was subjected to a current with a central carbon electrode where the chlorine was obtained and piped off. The workers who took care of the chlorine gas never developed chloracne thus refuting the original hypothesis by Herxheimer. By contrast, those who handled the electrodes and cleaned the reaction vessels were those afflicted with chloracne. Already at this time chlorinated phenolic compounds were considered as possible noxious agents (Fraenkel, 1902). This however could never be proven and even at present, when satisfactory analytical techniques are now available, no analysis of the so-called "tuffy tar" has been carried out.

Another class of chlorinated organic compounds causing skin damage appeared during the First World War (1914-1918). At this time perchlorinated naphthalenes had come into use as insulation materials, e.g., in the radio and electronic industry. The first description of Pernakrankheit was that by Wauer (1918). The use of the unspecified technical mixture of chlorinated naphthalenes spread all over the world and caused numerous intoxications notably among workers in manufacture. The perna disease has been summarized by von Wedel et al. (1943) and described in particular detailed by Braun (1955). Apart from chloracne the systemic effects of the same compounds have been dealt with by Drinker et al. (1937) and Greenburg et al. (1939).

Both in man and experimental animals, serious liver damage occurred after exposure to chlorinated naphthalenes, consisting of liver necrosis and toxic jaundice (acute yellow liver atrophy). Among several hundred cases of chloracne due to these compounds, Braun (1955) tabulated 24 deaths due to toxic jaundice and 14 recoveries. It should be pointed out that a fulminant liver disease with jaundice of this kind is an extremely rare condition. For comparison, it has never occurred after exposure to trichlorophenol (TCP) and TCDD as described below. Note should also be taken of the fact that not only were the perchlorinated naphthalenes an ill identified mixture of chemical species, but exposure frequently occurred at the same time to mixtures of chlorinated biphenyls, the latter now known to be contaminated with chlorinated dibenzofurans. The potentiation of toxicity by these mixtures and other chlorinated compounds were discussed by Drinker et al. (1937), Greenburg et al. (1939), von Wedel et al. (1943), and Risse-Sunderman (1959).

Several accidental ingestions of chloracnegenic compounds have

occurred. They are of particular importance in relation to discussions
on whether chloracne is a systemic or local disease. The so-called
Yusho disease is discussed in section 11.

Herzberg (1947) described several cases of chloracne, in which
other toxic signs and symptoms were seen, due to consumption of
"chlorinated paraffin" used as a substitute for butter during cooking
in postwar Berlin. Among general signs and symptoms observed were
gastrointestinal disturbances with abdominal pain, headache, pain in
joints, neuropathy, depression, and lack of appetite. The
dermatological symptoms were erythema, exanthema, comedones, and
retention cysts in sebaceous glands. It was noted as remarkable that
the skin signs had a follicular predilection, as in seborrhoea (face,
head, bosom, and back). The slow development of chloracne, and
particularily the fact that the sebaceous glands were affected, led
the author to conclude that it was a secretory disease
(Ausscheidungstoxikose). With regard to the chloracnegenic component,
it is unlikely that paraffin itself was active. Herzberg speculated
that something else, possibly a pyrolysis product that arose during
cooking, could have caused the disease.

Accidents in chemical plants involved in the manufacture of
chlorinated phenolic compounds are listed in Table 63. It should be
stressed that all these intoxications are due to mixtures, e.g., TCP
and TCDD and other compounds. Summaries of the industrial accidents
are to be found in Holmstedt (1980) and only some of them will be
dealt with here.

Table 63. Summary of accidents in chemical plants involving the manufacture
of chlorinated phenolic compounds

Country and date	Product[a]	Cause of exposure	Personnel affected	Years from incident to last observation	References
Germany 1910 Same year Dohmeier & Janson (1983)	CP	Explosion + occupational	5		Teleky (1913), Wahle (1914),

Country	Year	Product	Cause	No. exposed	No. affected	References
United States	1949	TCP	Explosion + Occupational	228	30	Ashe & Suskind (1949, 1950), Suskind et al. (1953), Huff et al. Zack & Suskind (1980) & Gaffey (1983), Moses et al. (1984), Suskind & Suskind (1978), (1980), Zack et Hertzberg (1984)
Federal Republic of Germany	1949	TCP (PCP)	Occupational	17	1	Baader et al. (1951)
Federal Republic of Germany	1952	TCP	Occupational	60		Bauer et al. (1961)
Federal Republic of Germany	1952–1953	TCP	Occupational	37		Hay (1977)
Federal Republic of Germany	1953	TCP	Explosion + Occupational	75	29	Hoffman (1957), Goldmann (1972, 1973), Huff et al. (1980), Thiess et al. (1982)
France	1953	TCP	Explosion + Occupational	17	2	Dugois & Colomb (1956, 1957), Dugois et al. (1958)
Federal Republic of Germany	1954	TCP, 2,4,5-T	Occupational	31	24	Schultz (1957a,b), Bauer et al. (1961), Kimmig & Schultz (1957a,b)

Kleu
& Göltz (1971), von Krause &

Brassow (1978)

Table 63 (contd - 2).

Country and date	Product[a]	Cause of exposure	Personnel affected	Years from incident to last observation	References
Federal Republic of Germany 1954	TCP 2,4,5-T	Occupational	24	4	Risse-Sundermann (1959)
United States 1956	2,4,5-T 2,4,5-T	Occupational	48	6	Bleiberg et al. (1964), Poland et al. (1971)
United States 1956	TCP	Occupational	Many		Hay (1977)
Italy 1959	TCP	Explosion + Occupational	5	2	Hofman et al. (1962)
United States 1959	TCP	Occupational			Hay (1977)
United States 1960	TCP	Occupational	Many		Hay (1977)
Netherlands 1963	TCP 2,4,5-T	Explosion	106	11	Dalderup, (1974a,b), Berlin et al. (1976), Huff et al. (1980)
USSR 1964	2,4,5-T	Occupational	128		Telegina et al. (1970), IARC (1977)

Country and date	Product[a]	Cause of exposure	Personnel affected	Years from incident to last observation	References
United States 1964	TCP	Occupational	61	6	Vahrenholt (1977), Cook et al. (1980), Ott et al. (1980)
Czechoslovakia 1964–1969	TCP	Occupational	80	6	Jirásek et al. (1973, 1976), Pazderova et al. (1974, 1980, 1981)
United Kingdom 1968	TCP	Explosion	90	14	May (1973, 1982), Huff et al. (1980)
Japan 1970	2,4,5-T	Occupational	25	3	Mivra et al. (1974)

Table 63 (contd - 3).

Country and date	Product[a]	Cause of exposure	Personnel affected	Years from incident to last observation	References
USSR 1972	TCP	Occupational	1	1	Zelikov & Danilov (1974)
Austria 1972-1973	2,4,5-T	Occupational	50		Forth (1977), Hay (1977)
Federal Republic of Germany 1974	2,4,5-T	Occupational	5		Forth (1977), Hay (1977)
Italy 1976	TCP	Explosion	193	8	Reggiani (1977, 1978, 1983a), Vahrenholt

(1977), Filippini

et

al. (1981), Abate et al.

(1982),

Ideo et al. (1982)

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a   Products: TCP = trichlorophenols; CP = chlorophenols; PCP
= pentachlorophenol;
        2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

The first reported intoxication with a mixture probably
containing TCDD, although the chemical structure was not given,
occurred in February 1910. Five people were said to have been
contaminated after a reactor explosion and two of these were described
in some detail in a dermatological thesis (Teleky, 1913; Wahle 1914).
Wahle (1914), however, in his thesis emphazised that this intoxication
was not due to any of the chlorinated naphthalene derivatives that
were well known by then.

An industrial poisoning was reported in 1949, due to the
formation of TCDD in uncontrolled exothermic reactions occurring
during the manufacture of TCP at a 2,4,5-T-producing factory in Nitro,
West Virginia, USA. The temperature in one of the reactors containing
tetrachlorobenzene, methanol, and sodium hydroxide increased, a relief
valve opened, and the contents of the vessel were discharged into the
interior of the building and over a wide area outside of the building.
A total of 228 people were affected.

Symptoms included chloracne, nausea, vomiting, headaches, severe
muscular aches and pains, fatigue, emotional instability, and
intolerance to cold. Laboratory findings showed an increase in total
serum lipids and an initially prolonged prothrombin time. Among those
affected were not only workmen, but also laboratory personnel, medical
personnel, and even the Safety Director who visited the area of
exposure. Several wives who had never visited the plant also developed
acne, usually at the same time as their husbands working at the plant.
A man from the nearby town who purchased a truck that was parked in
the vicinity of the accident at the time it occurred, and his child,
also developed chloracne. The disabling symptoms, which kept men from
their jobs for as long as 2 years, were severe aches and pains and
fatigability, the manifestations of peripheral neuropathy. Liver tests
4 years later were normal, but mild cases of acne were common. TCDD

was still an unknown chemical. The follow-up to this accident will be discussed in section 8.4.

In 1953, at the Badische Anilin and Soda Fabrik, during the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenol, the temperature and pressure in an autoclave increased rapidly and resulted in an exothermic reaction releasing a great deal of steam through a safety valve of the reaction vessel. This steam covered the walls, windows, doors, and machinery in the rooms of four floors, and finally precipitated in solid form on everything in these rooms. Forty-two workers involved in the clean-up operations developed chloracne, and even after the extensive clean-up operations occasional workers still developed chloracne. Thereafter the autoclaves were used for 2 years without incident but in 1958 a mechanic who conducted repair work on an autoclave subsequently developed chloracne (Hofmann, 1957; Goldmann, 1972). In 1968 and 1969 the building containing the autoclaves was dismantled. Goldmann (1972, 1973) conducted a study of the 42 workers exposed in this accident. In

21 cases, the chloracne was preceded by a non-specific dermatitis and in two cases very persistent chronic conjunctivitis and blepharitis were observed; 14 cases also showed involvement of other organs. In four instances the liver was affected, and microscopic examination of the liver again showed a very characteristic grey pigment that did not stain positive for iron. A transient involvement of the myocardium was also noted. In five instances the upper respiratory tract was involved with tracheitis and bronchitis. There was one instance of haemorrhagic pleuritis and one instance of afebrile gingivitis and stomatitis. In a number of cases a high susceptibility to infection was noted, sometimes accompanied by a decrease in gamma-globulin. One worker died of pancreatitis, in seven cases the central nervous system was affected, three instances of toxic polyneuritis were recorded, and in two instances hearing, sense of smell, and taste were impaired. The child of one of these workers also developed chloracne, and in most of the workers active chloracne persisted for many years - in one instance for 18 years. Follow-up studies are described in section 9.4.

Of particular interest is a study by Risse-Sundermann (1959). According to oral reports by the treating physician, all 24 members of a team working in a trichlorophenol operation became ill after the production process was switched to the pressurized phenol process in the spring and summer of 1954. Slightly different acneiform skin conditions appeared as symptoms of the toxic exposure. In addition, the patients suffered from dizziness, nausea, vomiting, lacrimation, burning of the eyes, difficulty in hearing, gastrointestinal spasms, intolerance to fatty foods, diarrhoea, jaundice, hepatitis (which was

fatal in one case), and paresthesias and hyperesthesias, as well as extreme irritability. One patient became psychotic and committed suicide. In addition, some of the patients complained of impotence.

Ten workers at this chemical factory were followed for five years by Risse-Sundermann (1959). In addition to the signs and symptoms mentioned above, she noticed swollen lymph glands and a considerable decrease in body weight. The patients underwent neurological examination, with no objective signs being observable. Of particular interest in this well documented study is the fact that in three patients the general symptoms (e.g., tiredness, depression, lack of appetite, stomach pains, sexual dysfunction) preceded that of the skin manifestations .

Bauer et al. (1961) reported a study of workers affected by three different outbreaks of chloracne. In this study more than 100 workers were examined. Of these, 31 Hamburg workers had been exposed 5 years ealier. Nine were examined in detail and their symptoms tabulated. Initially, there was dermatitis and irritation of the face, sometimes accompanied by conjunctivitis, and followed by the gradual development of chloracne and patchy pigmentation of the skin. In some cases irritations of the mucous membranes of the face and upper respiratory tract, together with a persistent blepharoconjunctivitis, were also noted. In the follow-up study, a number of cases of liver injury were

observed and, at liver biopsy, a typical grey pigment was observed in liver sections, which did not stain positive for iron. Viral hepatitis was suspected. In a few cases, chronic bronchitis and occasional myocardial damage were also observed. In all cases, fatigue was the main complaint and muscle weakness and muscle pain were described by the workers, particularly in the proximal muscles of the lower extremities. All nine also reported decreased libido. In a few instances, paresthesia and hyperesthesia or pronounced sensory neuropathy were observed, and minor circumscribed pareses were found. A psychovegetative syndrome occurred in most of the workers. Other signs recorded were: inability to concentrate, memory deficits, sleep disturbances, particularly increased somnolence, decreased drive, and alcohol intolerance. Psychological tests also showed abnormalities.

Following the malfunction of a reaction vessel in northern Italy, in which 2,4,5-trichlorophenol was produced, the temperature in the vessel increased rapidly and an intense black vapour filled the work-room covering everything with a black deposit. Five workers engaged in clean-up operations developed chloracne (Hofmann & Meneghini, 1962). None of those involved in the clean-up exhibited any involvement of general systemic toxicity (even after 16 months) that

could be related to exposure to the tar and soot. However, one
15-year-old worker developed folliculitis and superficial nodular
elements on the face a few days after initial exposure. A slow but
progressive generalization of the dermatosis developed on the trunk,
scalp, and lower extremities. An examination several months later
revealed no damage to the renal or liver parenchyma. However, this
worker was found to still suffer from outbursts of chloracne in 1980
(Holmstedt, 1980).

     Duverne et al. (1964) reported a case that occurred at a plant at
Lyon, France, where products that used 2,4,5-tri-chlorophenol as a
starting material were manufactured. This worker developed chloracne
as well as serofibrinous pleuritis.

     Ten workers also developed chloracne at a plant near Grenoble,
France, which produced 2,4,5-trichlorophenol that served as the
starting material for phenoxy pesticides and germicides for cosmetics.
These workers showed symptoms of systemic poisoning similar to those
reported by Goldmann (1972), and hepatic insufficiency with lipaemia
and elevated serum cholesterol levels (Dugois & Colomb, 1956). Another
accident resulting in TCDD exposure of workers occurred in the same
factory in 1966 (Dugois et al., 1967).

     An exothermic reaction resulted in an explosion at a plant in
Chesterfield, England, in 1968. The company made 2,4,5-trichlorophenol
from tetrachlorobenzene and the explosion occurred during the process
involving ethylene glycol and caustic soda under atmospheric pressure
(Milnes, 1971). In this incident, 79 workers developed chloracne but
there was no evidence of systemic illness (May, 1973). In 1971, 3
years after the explosion, two workers who had not been involved in

the explosion or its aftermath were employed as pipe-fitters at a new
installation, away from the site of the explosion, to refit one of the
cleaned tanks. They both developed severe chloracne, and the son of
one of these workers and the wife of the other also developed this
condition (Jensen & Walker, 1972). May (1973) cited two incidents
involving explosions in a similar process. In the first incident,
fatal injuries were recorded; in the second incident, all 50 exposed
persons fell ill after 10 days and had liver injury.

     In the USA, an outbreak of chloracne occurred among workers
manufacturing 2,4-dichlorophenoxyacetic acid and
2,4,5-trichlorophenoxyacetic acid (Bleiberg et al., 1964); 29 workers
developed chloracne and 11 of these had elevated urinary uroporphyrins
and exhibited varying degrees of acquired porphyria cutanea tarda. At
least one of these workers had abnormal liver-function tests and

microscopic examination of a liver biopsy specimen showed parenchymal cell regeneration and haemofuscin pigment. Many of the workers with chloracne showed hyperpigmentation of the skin. A second study of the workers at this plant was conducted in 1969 by Poland et al. (1971). A total of 73 male employees were examined, and moderate to severe chloracne was found in 13 workers (18%), mild chloracne in 35 (48%), hyperpigmentation in 30, and uroporphyrinuria in 1. No definite systemic illness could be documented in these workers. Of those studied, 33 had been employed at the plant for 0-4 years, 10 for 4-8 years, and 30 for more than 9 years. The mean duration of employment was 8.3 ± 7.6 years (mean ± 1 SD). The trichlorophenol manufactured in this plant contained 10-25 mg TCDD/kg. Twenty-six of the workers seen by Bleiberg et al. (1964) were also seen in a follow-up study. Six months prior to the second survey (Poland et al., 1971), the manufacturing process was altered so that the 2,4,5-T produced contained less than 1 mg TCDD/kg.

In 1964, in the USSR, many workers developed chloracne while engaged in producing 2,4,5-T. Production was then discontinued (Telegina & Bikbulatova, 1970).

On 10 July 1976, an explosion occurred at the ICMESA plant at Meda, near Seveso, Italy, when 12 workers were present. All 176 workers of the plant were examined 3 or 4 weeks after the accident. Chloracne was suspected in 1 of them; the others showed minor symptoms that could not be correlated with exposure. Alkaline phosphatase and delta-glutamyltransferase seemed slightly increased in 32 and 37 cases, respectively, while five workers showed a reduction in their delta-aminolevulinic acid dehydratase blood levels, and three showed moderately increased urinary gamma-aminolevulinic acid (Zedda et al., 1976). Similar findings were reported by Fara et al. (1976) and Reggiani (1978). The follow-up of the general population is described in section 8.2.

8.2 General Population Studies

8.2.1 Missouri, USA

Environmental exposures have occurred in a small area of Missouri, USA (Carter et al., 1975; Kimbrough et al., 1977; Kimbrough, 1984). In the summer of 1971 many birds, rodents, cats, dogs, insects, and horses died after exposure in a horse arena in eastern Missouri. The incident followed the spraying of "waste oil" on the horse arena for dust control. Within 3 weeks of the spraying of this arena, two other arenas were sprayed. In all, 57 adult horses died, 26 abortions occurred among the horses at the most heavily exposed farm and many

foals died soon after birth. At the time, the nature of the chemical that had caused the problem was unknown. The arenas were excavated and the contaminated dirt dumped at other sites. After many fruitless attempts to identify the cause of this outbreak, it was discovered in 1973-1974 that the original soil from one of the arenas contained 5600-6500 mg trichlorophenol/kg, 31.8-33.0 mg TCDD/kg, and 1350-1590 mg polychlorinated biphenyls/kg. Because of this finding, the episode was reinvestigated. It was found that the salvage oil company that sprayed the three arenas routinely collected discarded motor oil and lubricants from over 2000 service stations in eastern Missouri and southwestern Illinois. It also collected, from various sources, a limited amount of used organic solvents such as transformer oils and other compounds. A company in southwestern Missouri was finally identified as a source of TCDD. This company had manufactured trichlorophenol as an intermediate for hexachlorophene. The production of 2,4,5-tri-chlorophenol had generated a distillate residue which was emptied once a week into a residue storage tank. Initially this chemical waste was collected and incinerated but, in 1971 when the trichlorophenol producer experienced a financial crisis, he arranged for the chemical wastes to be disposed of by a chemical supplier. The chemical supplier subcontracted the chemical waste disposal to the salvage oil dealer. The salvage oil dealer added the toxic chemical waste to his salvage oil storage tank, having collected a total of 18 000 gallons. This material, mixed with salvage oil and other chemicals, was sprayed on the riding arenas and some of it was taken to re-refining companies. Soil samples from arenas where contaminated dirt had been dumped in 1974 contained trichlorophenol levels that ranged from 1.5-32.6 mg/kg, TCDD levels that ranged from 0.22-0.85 mg/kg, and polychlorinated biphenyl levels that ranged from 10-25 mg/kg.

A 6-year-old girl, who had used one of the arenas for sandbox-like play in 1971, developed epistaxis, headache, diarrhoea and lethargy, haemorrhagic cystitis, and signs of pyelonephritis. She had an uneventful recovery. Three other females exposed to the same arena had recurring headaches, skin lesions, and polyarthralgias. Two

3-year-old boys in another arena developed chloracne on the exposed skin surfaces which lasted for more than a year. Evaluation of the three female patients 5.4 years after exposure to TCDD-containing oil showed them to be in good health (Beale et al., 1977).

A comprehensive medical examination of 154 residents exposed to TCDD, and 155 unexposed residents in similar type housing in eastern Missouri, revealed no consistent differences between the two groups. The examination included a medical history, physical examination,

serum and urinary chemistries, and immunological and neurological
tests. The findings may suggest that long-term TCDD exposure is
associated with depressed cell-mediated immunity (decreased
delayed-type hypersensitivity skin reactions to standard antigens)
(Hoffman et al., 1986; Stehr et al., 1986). Urinary concentrations of
glucaric acid were not significantly different between persons
identified as being at high or low risk (Steinberg et al., 1985).

8.2.2 Seveso, Italy

     The scientific follow-up on the population of Seveso, N. Italy,
which had been accidentally exposed to TCDD in 1976 (see sections
4.1.1 and 8.1), was guided by an international steering group headed
by Professor M.A. Klingberg. The group completed its work in February
1984 and concluded that "it is obvious that no clear-cut adverse
health effects attributable to TCDD, besides chloracne, have been
observed" (Regione Lombardia, 1984). A total of 193 people had
displayed symptoms of chloracne, but at the beginning of 1984 only 20
presented active symptoms. After 15-20 days exposure to TCDD soil
levels of 270-1200 $\mu g/m^2$, there was a marked incidence of chloracne.
No disturbance of biochemical functions were seen when the exposure
had been limited to soil with TCDD levels at or below 30-70 $\mu g/m^2$.
Later evaluations failed to confirm earlier findings of a decrease in
motor nerve conduction velocity in some individuals. A significant
increase in urinary glucaric acid levels, indicating increased
microsomal enzyme activity, was found 3 years after exposure in 67
exposed children, as compared to 86 non-exposed children (Ideo et al.,
1982, 1985). The steering group found the data difficult to evaluate
as analytical and individual biological variabilities were not
explained (Regione Lombardia, 1984).

     Studies performed on the rate of spontaneous abortions and birth
defects in the Seveso area do not allow any conclusions to be drawn
(Tognoni & Bonaccorsi, 1982). The hypothesis that low exposure might
cause pre-pregnancy or pregnancy effects that adversely affect the
outcome was tested using several exposure models. The only finding was
a slightly higher rate of haemangioma among newborns in the exposed
group. However, this showed up only with one of the exposure models.
It was considered doubtful that this was due to TCDD exposure (Regione
Lombardia, 1984).

     Lymphocytes from inhabitants of Seveso were examined for
chromosomal aberrations by Regianni (1980a,b) and Mottura et al.
(1981). In 17 TCDD-exposed individuals examined within two weeks of
the accident, no increase in chromosomal aberrations was observed
(Regianni, 1980). In the abstract by Mottura et al. (1981),

chromosomal aberration analysis was performed on subjects distributed into three classes: acute exposure, chronic exposure, and a control group of non-exposed subjects. No significant difference in the frequency of chromosomal aberrations in the three exposure categories was reported. Data on number of subjects, chromosomal aberrations, and exposure level and time were not given.

Tenchini et al. (1983) published a comparative cytogenetic study on induced abortions from women exposed to TCDD after the Seveso accident, and in non-exposed subjects. Chromosome analysis was performed on maternal peripheral blood, placental and umbilical cord tissues, and fetal tissues. No significant differences were found in the level of chromosomal aberrations in the blood of placenta and umbilical cord from TCDD-exposed and non-exposed women. The exception was fetal samples from non-exposed women, in which a significant increase in chromosomal aberrations was obtained, possibly an artefact due to experimental techniques. The effect of TCDD on fetal chromosomes is therefore still unclear.

Several epidemiological follow-up studies are continuing in and around Seveso.

8.2.3 Viet Nam

From 1960 to 1969 a mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (Agent Orange), which was contaminated with TCDD (concentrations ranging from 0.5 to 47 mg/kg) (Kearny et al., 1972), was sprayed over areas of Viet Nam as a defoliant. The spraying from 1960 to 1965 was minimal; in 1966 it covered slightly more than 800 000 acres, in 1967 almost 1.7 million acres, in 1968 over 1.3 million acres, and in 1969 1.2 million acres. Studies have been carried out since the early 1970s to ascertain whether the exposure of the general population in Viet Nam to this herbicide could have resulted in an increased incidence of birth defects. However, the results of such investigations have not been published in readily available peer-reviewed journals, making it difficult to assess the scientific significance of the findings. Such studies have been reviewed by Westing (1984) and Constable & Hatch (1985). Those studies reviewed indicate a range of effects including spontaneous abortions, infertility, and birth defects. However, there are marked deficiencies in experimental design in most, if not all, studies, including potential bias in the selection of populations, poor record-keeping of populations and biological effects, such as congenital malformations, and a lack of control over possible

confounding factors. These deficiencies make it difficult, if not

impossible, to use this body of data in assessing the human health risks from exposure to phenoxyherbicides contaminated with TCDD and other PCDDs.

     Tung (1973) reported an increased incidence of liver tumours in Viet Nam. From 1955 to 1961 there were 159 cases of liver cancer out of a total of 5492 cancer cases, and from 1962 to 1968, 791 out of a total of 7911 cancer cases. Van (1984) continued Tung's investigation. Previous exposure to herbicides of 21 male cases of primary liver cancer and 42 controls was ascertained. Six of the 21 cases and three of the controls had lived or worked in areas sprayed with herbicides or had moved there shortly after spraying ceased. Residence time varied from 8 to 77 months. There is a lack of information on confounding factors and there was a chance for bias in these studies. In general, the possibility of exposure to multiple chemicals and the short latency period noted make the study by Van (1984) of little value for assessing risk (IARC, 1986).

     In 1979, the United States Air Force (USAF) initiated an epidemiological study into the possible health effects from chemical exposure of Air Force personnel who conducted aerial dissemination of herbicide in Viet Nam (Operation Ranch Hand) (Lathrop et al., 1984). The purpose of this investigation was to determine whether long-term health effects exist and can be attributed to occupational exposure to herbicides. This study used a matched cohort design in a non-concurrent prospective setting, incorporating mortality, morbidity, and follow-up studies. The report presented the results of health information on 2706 Ranch Handers and comparison individuals obtained by questionnaire and 2269 Ranch Handers and comparison individuals undergoing an extensive physical examination. It was concluded that there was insufficient evidence to support a cause and effect relationship between herbicide exposure and adverse health in the Ranch Hand group at this time. The study disclosed numerous medical findings, mostly of a minor or undetermined nature, that require detailed follow-up.

     In a study of 15 soldiers in Australia exposed to Agent Orange, no increases in structural chromosomal aberrations or sister chromatid exchanges were noticed, compared to a control group of 8 subjects (Mulcahy, 1980). In 1980 the Australian Commonwealth Institute of Health agreed to conduct a series of scientific investigations into the health of Viet Nam veterans and their families. After considerating the most appropriate study programme, it was decided in 1981 to conduct, as part of that programme, a case-control study of congenital anomalies and Viet Nam service (Donovan et al., 1984). The report is largely negative, as is that of Erickson et al. (1984) which

reported a similar study of American veterans.

Table 64. Signs and symptoms reported in association with human exposure
to TCDD or mixtures containing TCDD

_____

A. Skin Manifestations   C. Neurological Effects

1. Chloracne   1. Sexual dysfunction
2. Hyperkeratosis   2. Headache
3. Hyperpigmentation   3. Neuropathy
4. Hirsutism   4. Sight disturbance
5. Elastosis   5. Loss of hearing, taste,
                                              and smell

B. Systemic Effects   D. Psychiatric Effects

1. Mild fibrosis of liver   1. Sleep disturbance
2. Raised transaminase values   2. Depression
   in blood   3. Loss of energy and drive
3. Hypercholesterolaemia   4. Uncharacteristic bouts of anger
4. Hypertriglyceridaemia
5. Loss of appetite and weight loss
6. Digestive disorders (intolerance to
   alcohol or fatty food, flatulence,
   nausea, vomiting, diarrhoea)
7. Muscular aches and pains, joint
   pain, lower extremity weakness
8. Swollen lymph glands
9. Cardiovascular, urinary tract,
   respiratory, and pancreatic disorders

_____

8.3 Signs and Symptoms in Humans Associated With TCDD Exposure

     Many signs and symptoms have been reported in studies of human
exposures to PCDDs, both occupationally and from the general
environment. These have been compiled from the various studies and are
shown in Table 64.

8.3.1 Skin manifestations

Chloracne is a sign of exposure to several chlorinated cyclic organic compounds, the most potent being TCDD. Chloracne thus may serve as a marker of such exposure. The most distinctive lesion in chloracne is the so-called cyst, a skin-coloured elevation that may measure from 1 mm to 1 cm in diameter, with a central opening that may be difficult to detect. Comedones that contain black or black-appearing material in their openings are also present. There may

be a secondary inflammatory reaction, melanosis, and hyperkeratosis, and these skin changes may be preceded by a "cable rash" or "cable itch". These skin lesions resemble photosensitivity reactions and the bearers may suffer severe pruritus. Microscopic examination of the skin lesions shows marked dilatation of the hair follicles which are filled with keratinous material, the sebaceous glands may be partly or completely atrophied and, occasionally, hyperplasia of these glands has also been reported. Hyperkeratosis and acanthosis of the surrounding epidermis usually accompany these lesions. Atrophy of the epithelium and thinning of the epithelial walls surrounding these keratinous cysts are observed at a later stage of the disease. If the follicular cysts rupture, foreign body granulomata may also be observed. Healing of these skin lesions usually results in deeply pitted scars. The distribution of chloracne is predominantly facial, affecting in particular the malar areas, the jaws, and the regions behind the ears. At times it may involve the ear canal and, with increasing severity, also the rest of the face and neck. In more extensive cases, the outer upper arms, neck, back, abdomen, outer thighs, and genitalia may also be involved (Crow, 1970).

While the absence of chloracne does not absolutely rule out exposure to TCDD, it usually indicates that there has been no exposure to a toxic dose of the substance. "Toxic" is used here to indicate both systemic and local effects. Where there has been exposure to TCDD and chloracne has resulted, it is the only known clinical sign that persists for a long period of time, even for the remainder of the exposed person's life time. In a large group of people exposed to mixtures containing TCDD, the absence of chloracne usually indicates that exposure to a toxic dose was unlikely and also makes it unlikely that severe, persistent systemic disorders will result.

Hyperkeratosis is a fairly common phenomenon whereas hyperpigmentation and hirsutism are rare. It should be noted that hyperkeratosis is prominent in the exposed Seveso children who have no affected sebaceous glands. These glands develop only at puberty.

Elastosis of the skin has been noted as a long-term effect of TCDD exposure.

8.3.2 Systemic effects

     Liver effects following exposure to PCDDs have been diagnosed
even by histological examination, and account for temporarily raised
transaminases in blood, hypercholesteraemia, and
hypertriglyceridaemia. Bauer et al. (1961) and Risse-Sundermann (1959)
do not however exclude viral hepatitis as a cause of such findings in
their patients exposed to TCDD. Loss of appetite, weight loss, and
digestive disorders are common complaints from humans exposed to
either TCDD itself, or to technical mixtures containing TCDD. Muscular
aches and pain and weakness in extremities have been reported,
particularly after exposure to technical mixtures containing TCDD.

Swollen lymph nodes have also been reported, both after exposure to
"pure" TCDD and to mixtures. The cardiovascular, urinary tract,
respiratory, and pancreatic disorders reported are of doubtful
significance with regard to a causal relationship to TCDD exposure.

     Porphyria cutanea tarda has been reported in two cases of
occupational exposure where chlorinated organic compounds were
manufactured in addition to trichlorophenol. These were the incidents
at the factory of Diamond Alkali, Newark, New Jersey, USA, in 1956
(Poland et al., 1971) and at Spolana, Czechoslovakia, between 1964 and
1969 (Pazerova-Vejlupkova et al., 1981). The porphyria cutanea tarda
observed in these cases was very unlikely to have been induced by
exposure to TCDD but rather by exposure to other chlorinated organic
compounds manufactured in these plants (Jones & Chelsky, 1986).

8.3.3 Neurological effects

     Sexual dysfunction (lack of libido and impotence) has been
reported after acute exposure to both "pure" TCDD and technical
mixtures (Schulz, 1968). The frequency of its occurrence may have been
underestimated to date. Headache is a frequent symptom after exposures
to technical mixtures containing TCDD.

     Sensory neuropathy has been noted in many instances. Usually
workers in the initial stages of exposure will complain of pains in
their joints after they have very acute severe chloracne; however,
there are usually no abnormal physical findings in the joints, but the
complaints may continue. In early studies of workers affected by TCDD,
no attempts were made to objectively measure the effects on the
sensory nervous system. Tests have now been developed that evaluate
sensory nerves and that can be used in future field studies. The nerve
conduction tests, which primarily have been used so far, are actually

not very useful to measure neuropathy. Differences in nerve conduction were shown among residents from Seveso, Italy, who had chloracne and those who did not (Richert, von, 1962; Fillipini et al., 1981).

Sight disturbance may be related to alkaline exposure or to conjunctivitis related to effects on the glands of Meibom. Loss of hearing, taste, and smell have been reported in a few cases, but a causal relationship to TCDD exposure is doubtful.

8.3.4 Psychiatric effects

The symptoms have been listed in Table 64 in what is believed to be their order of frequency and degree of severity.

8.4 Epidemiological Studies

Signs and symptoms related to accidental exposure to TCDD are given in Table 64. However, it should be observed that all the accidents and occupational contamination concern exposure to a mixture of compounds where TCDD was only one component. In all cases, its concentration in the mixtures was unknown. Only two cases of intoxication with "pure" TCDD have been reported.

The story of the discovery of TCDD is by now well documented (Holmstedt, 1980; Sandermann, 1984a,b). TCDD was synthetized in 1955. Four people were intoxicated, one co-worker severely so while drying crystals. In all cases, decreased libido was the first symptom, followed by other symptoms such as moderate to severe chloracne, sleeping difficulties, inability to concentrate, depression, and, in at least one case, swelling of the lymph nodes. In all cases, the signs and symptoms disappeared within a couple of years, with the exception of the chloracne in the most heavily exposed man.

The second occasion of exposure to what one must assume to be pure TCDD is the one reported by Oliver (1975). The toxic effects on three young scientists who suffered "transient minimal exposure to TCDD" were described. Two of them suffered from typical chloracne. Delayed symptoms about two years after initial exposure occurred in two of the scientists. These symptoms were said to have included personality changes, other neurological disturbances, and hirsutism. All three scientists were found to have raised serum cholesterol levels, but no other biochemical disturbances and no porphyrinuria or liver damage were demonstrated. Whether the unusually delayed physiological effects were in fact due to the initial dioxin exposure is a question that was discussed by the author. Although conclusive evidence is lacking, it seems likely that these delayed effects were

in fact due to dioxin intoxication. The conditions of exposure remain unexplained.

Of the many cases of exposure reported in Table 63, only two (at Monsanto in 1949 and at BASF in 1953) have been adequately followed up epidemiologically with matched control groups.

The workers of Monsanto, USA, have been investigated several times between 1949 and 1984. Immediately after the accident, Ashe & Suskind (1949) hospitalized and studied four cases of severe poisoning among the workers. These four workers were diagnosed as having chloracne, but by the time of examination these men had recovered from earlier symptoms of peripheral neuropathy. In 1950, a further examination of these four workers and two additional men revealed continued irritability, nervousness, and insomnia (Ashe & Suskind, 1950). A consistent loss of libido and some impotence was reported. Further clinical examination revealed hepatomegaly, altered prothrombin times, and disturbed lipid metabolism.

A further study of 36 workers from this plant was undertaken in 1953 (Suskind et al., 1953). It was noted that even those who developed, to a moderate or severe degree, the skin eruptions, pains in back, dyspnoea, fatigue, nervousness, and decreased libido generally improved. Even those suffering the most severe cutaneous eruptions initially had only a few or no lesions in 1953.

More recent studies on these workers are those of Zack & Suskind (1980) and Zack & Gaffey (1983). Zack and Gaffey reported on a 121-member study cohort, with a presumptive high-peak exposure to TCDD base on chloracne occurrence, which was followed for mortality until 1978. The entire cohort was traced; there were 32 deaths; 89 people were still alive. There was no excess in total mortality or in deaths from malignant neoplasms. The proportional mortality analysis of decedents according to exposure by 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) indicated no unusual patterns of mortality. The proportional mortality ratio (PMR) for malignant neoplasms was low (PMR = 82) in the exposed group. Lung cancer was the only site among the malignant neoplasms for which the value was somewhat higher in the exposed group.

The Monsanto workers were again examined in 1984 (Suskind & Herzberg, 1984). A clinical epidemiological study was conducted to determine the long-term health effects of workplace exposures during the process of manufacturing the herbicide 2,4,5-T, including contaminants such as TCDD. The population consisted of two cohorts, 204 clearly exposed and 163 not exposed (controls). Among the exposed

workers, clinical evidence of chloracne persisted in 55.7%. None of the controls experienced chloracne development. An association was found between the persistence of chloracne and the presence and severity of actinic elastosis of the skin. There was an association between exposure and the history of ulcers of the gastrointestinal tract. Pulmonary function values among those who were exposed and who currently smoked were lower than those who were not exposed and who currently smoked. No disturbances of sexual functions were found at this time after age adjustment. The data assembled in the study indicated no evidence of increased risk for cardio-vascular disease, hepatic disease, renal damage, or central or peripheral nervous system problems.

Another selection of the population from the same plant has been examined by another group of epidemiologists (Moses et al., 1984). Since the degree of exposure was unknown to these investigators and since chloracne is generally considered a quite reliable indicator of heavy dioxin exposure, it was decided to use chloracne as a "surrogate" for exposure and to classify the study population by its presence or absence. It was recognized that those without chloracne, but with appropriate work-exposure history, might also have had TCDD exposure and were not therefore used as "unexposed controls".

Chloracne was found in 52% of 226 workers in a 1979 cross-sectional survey at the plant where 2,4,5-T had been manufactured from 1948 to 1969. Mean duration of residual chloracne was 26 years, and in 29 subjects it had been present for 30 years. A significantly increased prevalence of abnormal gamma-glutamyl transpeptidase (GGT) and higher mean GGT were found in those with chloracne compared to those without. Although mean triglyceride values were higher in those with chloracne, the difference was not statistically significant. Neurological examination showed a statistically significant higher prevalence of abnormal sensory findings in those with chloracne. Increased prevalence of angina and reported myocardial infarction in those with chloracne was not significant when age-adjusted. Increased prevalence of reported sexual dysfunction and decreased libido in those with chloracne, compared to those without, was statistically significant after age adjustment. No differences were found between those with and without chloracne in serum cholesterol, total urinary porphyrins, or in reproductive outcomes. Exposure to TCDD in 2,4,5-T production may thus result in apparently permanent changes in the skin. Sensory changes in peripheral nerves and possible changes in liver metabolism in those with current or past chloracne are also suggested by these data. Based on worker histories, even severe acute toxicological effects of TCDD are reversible, or improve markedly over time. While the cross-sectional nature of this study, the low participation rate,

and the highly select nature of the population limit the conclusions
that can be drawn, it is unlikely that permanent, severe, and
debilitating toxicological sequelae are inevitable after exposure to
TCDD sufficient to produce chloracne. It must be noted, however, that
individual susceptibility may make certain workers with heavy
exposures more vulnerable.

The exposure of workers at BASF in 1953 has been the subject of
several reviews, the latest one being that of Thiess et al. (1982).
Twenty-seven years after the accident that occurred in the BASF
Ludwigshafen plant, a mortality study was undertaken of people exposed
in the uncontrolled reaction which occurred during the trichlorophenol
process. The follow-up was 100% successful and involved 74 people.
Overall mortality (21 deaths) did not differ in this group from the
rate expected in three external reference populations, or from that
observed in two internal comparison groups, where 18-20 deaths were
observed. Of the 21 deceased people, 7 had had cancer, compared with
4.1 expected. In addition, two other cases of cancer (one bronchial
carcinoma and one carcinoma of the prostate) were still alive at the
time of writing. Three deaths due to stomach cancer at ages 64, 66,
and 69 years, were found, compared with 0.6 expected from regional
mortality data. One stomach cancer occurred among 148 individuals in
the two comparison cohorts. The incidence of cancer in these workers
was considerably greater than expected and cannot be explained only as
a chance event. Of 74 people, 66 had severe chloracne or severe
dermatitis. There is a possibility that some members of the BASF
cohort were exposed to other unknown occupational hazards before or
after the accident. However, the use of two internal comparison groups

composed of matched controls from the same factory was designed to
control for, as far as possible, other occupational exposures that
could be important etiological or confounding factors. Because of the
small size of the cohort and the small absolute number of deaths from
any particular cause, the results of this study do not permit any
definite conclusions concerning the carcinogenic effect of exposure.

In comparison with the above-mentioned, well conducted long-term
epidemiological studies, a host of other follow-up studies have been
published, none of which used adequate controls. They are, therefore,
of less value but will be briefly summarized here.

Jirasek et al. (1973, 1976) and Pazderova et al. (1974, 1980,
1981) examined 55 of a total of 80 workers who suffered intoxication
during the manufacture of sodium pentachlorophenate and the sodium
salt and butyl ester of 2,4,5-T. One worker died from severe acute
intoxication at an early stage (Jirasek et al., 1976), and 76 workers

developed chloracne. The following additional symptoms were found:
porphyria cutanea tarda, disorders of the metabolism of lipids,
porphyrins, and carbohydrates, and alteration of plasma proteins.
Hepatic lesions were also present. Neurological and electromyographic
(EMG) examinations revealed peripheral nerve changes in 17 people,
first detected in 8 people during the second year of the study. A
neurasthenic syndrome was also observed. The patients with porphyria
cutanea tarda showed hyperpigmentation, hypertrichosis, and bullosis
actinica mechanica. Porphyrin excretion in urine ranged from 172 to
2230 µg/24 h. Polyneuropathies, confirmed by EMG examination, were
noted, predominantly in the lower extremities. In this outbreak, the
disease was progressive during the first 2 years; subsequently the
dermatological symptoms as well as the porphyric disease and the
neurological disorders improved. The impaired lipid metabolism
improved only very slowly.

In this plant, the toxic substances were led off through the
breathing zone of the workers. The concentrations of the chlorinated
hydrocarbons in the air were never measured. Due to insufficient data,
the real hygienic conditions at the work place could not be accurately
reconstructed. The manufacturing of 2,4,5-T was halted permanently in
1968 so that it was impossible to obtain the necessary information in
an adequate manner. From 1959 to 1964, according to information from
the plant, only sodium pentachlorophenate was manufactured. Not until
1965 was the manufacture of sodium 2,4,5-T commenced on a pilot scale,
and later the butyl ester of 2,4,5-T was also manufactured. After each
year of production, something was always changed or modified in the
process and technology so that actually there was never full-scale
production in the true sense of the word. Many of the herbicides
manufactured could not be found from the documentation (Pazderova et
al., 1974). The uncertain mixture of compounds involved in the Spolana
episode makes interpretation of signs and symptoms almost impossible.
In all likelihood the porphyria observed was due to the
hexachlorobenzene stated to be produced at this factory.

Signs of disturbance in the porphyrin metabolism in workers
manufacturing 2,4,5-T was also described by Poland et al. (1971).
Chloracne was not correlated significantly with job location within
the plant, duration of employment, or coproporphyrin excretion.
Although 11 subjects with uroporphyria and at least three with overt
porphyria cutanea tarda had been found in a study of the same plant
six years earlier (Bleiberg et al., 1964), no clinical porphyria could
be documented at the time of the second investigation, and only one
worker had persistent uroporphyrinuria. Evidence of toxicity in other
organ systems was markedly less than that reported in previous studies
and in most instances there was no difference from normal populations.

In all likelihood the porphyria cutanea tarda in this case, as in the study from Czechoslovakia, was due to a compound other than TCDD. This is corroborated by a recent re-evaluation of the literature (Jones & Chelsky, 1986).

A study from northern Germany was published by Bauer et al. (1961). It is not clear where the cases orginated and only nine patients were studied in depth. A summary of the findings from these patients, and another person suffering from chloracne after occupational exposure to trichlorphenol, was reported by Kleu & Göltz (1971). These patients were followed for 15 years after exposure. The severity and types of symptoms varied in a dose-related manner. Major complaints were decreased sexual activity, muscular weakness, easy fatigability, irritability, and loss of appetite and memory. The authors concluded that a permanent defect had occurred, the late form of which resembled a cerebral involutionary syndrome, combined with mental depression and neurasthenia.

A follow-up study of 11 of the 24 employees at Boehringer exhibiting skin lesions in 1955 was published by von Krause & Brassow (1978). Many continued to suffer from their earlier complaints. In seven of the eleven, nausea and intolerance to heavy fatty food was still common, and six men complained of alcohol intolerance. Although conjunctivitis had disappeared, chloracne was still clearly visible in most of the 11 subjects. Neurological problems were still severe in six of the workers.

Ten years after the incident at Coalite in 1968 when 79 workers developed chloracne due to exposure to a chlorophenol-TCDD mixture, a study was undertaken to establish the state of health of the affected employees (46) remaining in the company's employment (May, 1982). Forty-one of the 46 employees participated. The opportunity was used to examine effects on mortality, morbidity, carcinogenesis, reproduction, teratogenicity, fetotoxicity, biochemistry, immunology, and genetic changes. Concurrently, two control groups were established, one with no dioxin exposure and the other with possible dioxin exposure. These groups were selected from within the works and matched the study group with respect to sex and age, but it was not

possible to match them for occupation and social status. Half the affected subjects still had minor chloracne. Other than this finding, the authors concluded that the subjects had not been had been adversely affected in any way.

Data on the mortality of workers at the Dow Chemical company have been provided in two papers (Cook et al., 1980; Ott et al., 1980). The

first of these studies describes the mortality of a cohort of 61 males
involved in the preparation of trichlorophenol. Forty-nine of these
workers developed chloracne, presumably as a result of skin absorption
of the process contaminant TCDD. Within the limitations posed by
cohort size and length of follow-up, the exposure to chlorophenol-TCDD
mixtures did not appear to have adversely affected mortality
experience. Overall, four deaths occurred and 7.8 were expected. Of
these, one death was due to cardiovascular disease (3.8 expected) and
three deaths were attributed to cancer (1.6 expected). None of the
findings was statistically significant. The second paper examined the
mortality experience of 204 people exposed to 2,4,5-T during its
manufacture from 1950 to 1971. Length of employment within the 2,4,5-T
process area ranged from less than one year to a maximum of
approximately ten years. Efforts to minimize TCDD contamination of the
product resulted in non-detectable concentrations (less than 1 mg/kg)
near the end of this period. Within the scope of this mortality
survey, no adverse effects were observed with respect to occupational
exposure to 2,4,5-T or to its feedstock, 2,4,5-trichlorophenol.

     Hardell and his co-workers in Sweden have conducted a series of
case-control studies and reported an increased risk of soft-tissue
sarcomas in men who were exposed to phenoxy herbicides and/or
chlorophenols (Hardell & Sandsstrom, 1979; Hardell, 1981; Hardell et
al., 1981; Hardell & Ericksson, 1981). These authors also reported a
case-control study that suggested that phenoxyacetic acids and
chlorophenols may predispose to Hodgkin's lymphoma (Hardell et al.,
1981). The relative risk was higher for a group exposed to phenoxy
herbicides including 2,4,5-T and chlorophenols, i.e., pesticides that
may be contaminated with PCDDs and PCDFs. However, an increased risk
was still found in a group exposed mainly to phenoxy herbicides such
as MCPA, 2,4-D, mecoprop and dichloroprop, i.e., pesticides with low
or no contamination with PCDDs and PCDFs.

     Analysis of fat levels of PCDDs and PCDFs in patients with soft
tissue sarcomas and in controls failed to reveal any differences
between the two groups (Nygren et al., 1986) (section 4.4.4.1).

     A cohort study on Swedish farmers and gardeners has been carried
out recently (Wiklund & Holm, 1986). Despite the greatly increased use
of phenoxyacetic acid herbicides from 1947 to 1970, no time-related
increase in the relative risk of soft-tissue sarcoma was found in the
cohort or in any of the subcohorts. The same was found by Hoar et al.

(1986) although the latter study points to an increase in non-Hodgkin
lymphoma. It should be noted that in all these studies the majority of
the herbicides used did not contain TCDD.

In follow-up studies of workers exposed to 2,4,5-T and its precursor 2,4,5-trichlorophenol (and therefore, presumably, also to TCDD), no excessive deaths due to any cause were registered (Cook et al., 1980; Ott et al., 1980; Zack & Suskind, 1980; Zack & Gaffey, 1983).

Honchar & Halperin (1981) merged the above four cohorts and found that three (2.9%) of the total 105 deaths were reported to be from soft-tissue sarcoma. Based on national statistics only 0.07% was expected to be due to this cause. Fingerhut et al. (1984) reviewed the employment records, medical and pathological reports, tissue specimens, and death certificates for these three cases and four additional cases of deaths from soft-tissue sarcomas in these and related cohorts reported by Cook (1981), Moses & Selikoff (1981), and Johnson et al. (1981). Three out of the seven cases had a record of chloracne and one of dermatitis. After review of the tissue specimens, five of the seven cases were diagnosed as soft-tissue sarcoma. The remaining two (which were among the three cases in the merged cohort of Honchar & Halperin (1981)) were found to be carcinoma. For three of the cases with confirmed soft-tissue sarcoma the exposure was not well documented, although an undocumented contact with 2,4,5-T, 2,4,5-trichlorophenol, or TCDD could not be excluded.

8.5 Human Experimental Studies

Poiger & Schlatter (1986) studied a human volunteer after ingestion of a single dose of 1.14 µg ^{3}H-TCDD/kg body weight. The absorption from the intestine was > 87% and adipose tissue levels were 3.09 (± 0.05) and 2.85 (± 0.28) ng/kg after 13 and 69 days, respectively. The estimated half-life of TCDD was 2120 days.

Gorski et al. (1984) calculated the half-lives of 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD and octaCDF to be about 3.5, 3.6 and 2 years, respectively. The estimation was based on the analysis of fat tissue biopsies collected with an interval of 28 months from one 14-year-old girl who for a period of about 2-3 years had been exposed to technical pentachlorophenol. Analysis was performed by gas chromatography with electron capture detection, and the isomers were confirmed by the use of several different packed and capillary columns.

9. TOXICOKINETICS OF PCDFs

9.1 Uptake, Distribution, and Excretion

Toxicokinetic data for TCDF and other PCDFs arise from iv injections or gastrointestinal exposure. There are no studies on exposure via the respiratory tract or via dermal application.

9.1.1 Studies with 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF)

Table 65 summarizes the distribution of radioactivity to the major tissue depots at various time-points after an iv injection of ^{14}C-TCDF into rats and mice. Similar studies in guinea-pigs and monkeys are summarized in Table 66. Tables 67 and 68 give the tissue distribution of ^{14}C-TCDF in more detail for rats and mice, respectively.

TCDF has been used for kinetic studies in the rat (Birnbaum et al., 1980), mouse (Decad et al., 1981a), guinea-pig (Decad et al., 1981b), and monkey (Birnbaum et al., 1981). A single iv dose of 30.6 µg ^{14}C-TCDF/kg body weight was given to rats, mice, and monkeys, while the guinea-pigs received an iv dose of 6 µg/kg body weight. The distribution of the radio-label was followed in tissues and excreta for 3 weeks in rats and monkeys, for 10 days in mice, and for 9 days in guinea-pigs. The distribution of radioactivity in the main tissues and excreta of the different species at some of the intervals studied is presented in Tables 65 and 66 along with the respective half-lives and LD_{50} values for TCDF. Radioactivity recovered from the tissues represented the parent compound, while radioactivity in faeces and urine represented metabolites of TCDF. In the faeces of guinea-pigs only the parent substance was present. Analysis by thin-layer chromatography revealed Rf values of 0.5 and 0.1 for metabolites of TCDF in faeces and urine as compared to an Rf of 0.8 for the parent compound.

TCDF has a short half-life (2-4 days) and is quickly eliminated from the liver, both in the rat and the mouse (Birnbaum et al., 1980; Decad et al., 1981a). Elimination occurs rapidly also from the skin and muscle, whereas retention is longer in adipose tissues. The difference in the retention of TCDF in adipose tissues between C57Bl/6 and DBA/2 mice may be explained by the fact that DBA/2 mice have substantially more adipose tissues than C57Bl/6 mice.

The distribution of TCDF in the guinea-pig was different from that in the rat or mouse (Decad et al., 1981b). The maximum uptake in the liver occurred within one hour after dosing; thereafter the radioactivity was distributed in the fat and skin during the succeeding hours. After one day, as a result of loss of body fat, the radioactivity in adipose tissues was redistributed to the liver.

Within 3 days after dosing there was no elimination of radioactivity from the liver and adipose tissues, whereas in the skin radioactivity decreased only slightly. The estimated half-life for TCDF in the guinea-pig was more than 20 days.

Table 65. LD_{50}, whole-body half-life, and distribution of radioactivity (percentage of administered dose) at various intervals after an iv dose of 30.6 µg ^{14}C-TCDF/kg to rats[a] and mice[b]

	Fisher 344 Rats			DBA/2 Mice			C57BL/6 Mice		
	3 hr	3 days	10 days	3 h	3 days	10 days	3 h	3 days	10 days
Liver	41.4±3.6	5.9±0.3	1.3	51.0±13.4	22.7±1.8	1.1±0.3	39.4±0.6	16.8±1.4	5.6±2.0
Fat	10.0±1.0	11.1±2.3	1.8	6.0±1.6	2.9±2.1	ND	9.6±3.9	22.3±2.9	7.2±0.9
Skin	6.6±0.3	1.2±0.3	0.5	3.6±0.7	3.0±1.1	ND	5.5±4.3	3.3±0.9	ND
Muscle	5.9±0.4	0.3	< 0.3	7.5±2.8	1.5±0.9	ND	10.8±3.4	5.4±1.9	1.8±0.4
Faeces		63.1±0.6	> 85		43.1	81.9±13.0		27.7	55.8±4.8
Urine		2.0±0.4	< 6		7.7	12.6±0.1		9.2	19.9±4.6
Half-life (days)		< 2[b]			2[b]			4[b]	
LD_{50} (µg/kg)		> 1000[c]			> 6000[c,d]				

[a] Birnbaum et al. (1980).

[b] Decad et al. (1981b).

[c] Moore et al. (1976).

[d] Moore et al. (1979).
ND = not detectable.

Table 66. LD_{50}, whole-body half-life, and distribution of radioactivity (percentage of administered dose) at various intervals after an iv dose of ^{14}C-TCDF in guinea-pigs[a] and monkeys[b]

	Hartley guinea-pigs			Rhesus monkeys
	3 h	3 days	9 days	21 days
Liver	23.6±3.8	29.3±0.6	54.2±14.5	1.02±0.80
Fat	31.4±0.7	56.9±7.6	21.8±11.6	3.66±2.83
Skin	22.5±0.1	17.1+±0.6	15.2±3.1	2.44±1.60
Muscle		15.6±4.5	8.8±3.0	1.55±0.14
Faeces		4.7±1.3	6.6	42.9
Urine		2.3±0.4	6.6	7.9
Half-life (days)		> 20[c]		8[a]
LD_{50} (μg/kg)		> 5[c] < 10[d]		1000[d]

[a]   6 μg/kg (Decad et al., 1981a).
[b]   30.6 μg/kg (Birnbaum et al., 1981).
[c]   Moore et al. (1976).
[d]   Moore et al. (1979).

Table 67.   Tissue distribution of TCDF-derived radioactivity in Fisher 344 rats at 15 min, 3 h, and 24 h following a single iv dose of 30.6 μg ^{14}C-TCDF/kg [b]

Tissue	Tissue content of [14]C (% of dose/g tissue)[a]		
	15 min	3 h	24 h

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Blood          0.12±0.04      0.04±0.01      0.03±0.01
Liver          4.4 ±0.2       5.1 ±0.4       2.2 ±0.4
Fat            0.20±0.03      0.44±0.07      0.64±0.11
Muscle         0.25±0.01      0.06±0.00      0.30±0.02
Skin           0.17±0.02      0.20±0.01      0.07±0.01
Kidneys        0.67±0.04      0.17±0.03      0.08±0.01
Adrenals       7.4 ±6.9       4.7 ±1.3       0.34±0.14
Thymus         0.52±0.12      0.54±0.13      0.07±0.03
Spleen         0.37±0.07      0.08±0.02      0.02±0.00
Testes         0.09±0.01      0.09±0.01      0.06±0.02
Brain          0.25±0.01      0.15±0.03      0.02±0.00
Lungs          1.08±0.08      0.24±0.02      0.07±0.02
Heart          0.66±0.03      0.11±0.00      0.02±0.02
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[a]   Mean ± SD for three animals.

[b]   From: Birnbaum et al. (1980).

     Based on data from three monkeys, the half-life for TCDF was
calculated to be 8 days (Birnbaum et al., 1981). At the end of the
study, more radioactivity remained in adipose tissues and skin than in
the liver. The retention of TCDF in the liver of monkeys 21 days after
dosing was comparable to that in the liver of the rat and C57Bl/6
mouse 10 days after injection. Urinary elimination of radioactivity
was a minor route when compared to faecal elimination both in the rat,
mouse, and monkey, whereas in the guinea-pig these routes were of
comparable importance (Birnbaum et al., 1980, 1981; Decad et al.,
1981a,b). The cumulated excretion of radioactivity 3 days
post-treatment amounted to approximately 64, 51, 11, and 7% in the
rat, C57Bl/6-mouse, monkey, and guinea-pig, respectively.

     Against this background of data on tissue distributions,
half-lives, and LD_{50} values of TCDF in the rat, guinea-pig, and
monkey, Birnbaum et al. (1980, 1981) concluded that TCDF, measured as
excreted radioactivity, is metabolized to less toxic compounds and
that animal species with a high capacity to metabolize TCDF are more
resistant to its acute toxicity. This conclusion was considered
applicable also to the mouse (Decad et al., 1981a). Based on the same
data King et al. (1983) produced a pharmacokinetic model for TCDF in
rats, mice, and monkeys. However, there are objections to this
comprehensive conclusion. First, the kinetic studies on guinea-pigs
(Decad et al., 1981b) were (for analytical reasons) carried out with
such a high dose of TCDF that all of the animals showed marked signs
of toxicity, even within 3 days. After 9 days all the animals were
killed due to toxic symptoms. It is not advisable to draw any

conclusions on normal kinetic behaviour from data obtained on dying
animals with their abnormal metabolism and physiology. As far as the
kinetic data from the monkey are concerned, the conclusions were based
on a single time-point, and the number of animals in that study was
also very limited (Birnbaum et al., 1981).

Table 68. Tissue distribution[a] of TCDF-derived radioactivity in C57Bl/6
and DBA/2 mice at 15 min, 3 h, and 24 h after
a single iv dose of 30.6 μg ^{14}C-TCDF/kg [b]

Tissue	Tissue content of ^{14}C (% of dose/g tissue)[a]					
	15 min		3 h		24 h	
	C57Bl/6	DBA/2	C57Bl/6	DBA/2	C57Bl/6	DBA/2
Blood	1.1	ND	0.6±0.3	0.22±0.04	ND	0.2±0.0
Liver	28.0±4.2	30.7±2.8	39.3±2.8	38.0 ±3.8	25.3±4.2	19.2±1.9
Adipose	2.6±0.4	2.7±1.0	3.7±1.4	4.9 ±0.1	6.1±1.2	6.1±0.4
Muscle	1.3±0.1	1.6±0.2	0.7±0.2	0.9 ±0.3	0.3±0.1	0.8±0.1
Skin	2.2±0.1	2.3±0.7	1.4±0.2	2.7 ±0.6	1.3±0.7	2.7±0.6
Kidneys	3.4±0.3	4.1±0.5	1.1±0.3	1.3 ±0.3	0.6±0.1	0.7±0.2
Adrenals	18.8±4.9	ND	6.9 ±5.4	9.2	6.5	ND
Thymus	3.9±2.4	3.0±1.9	2.0±0.7	4.7 ±3.2	0.3±0.2	2.5±0.6
Spleen	1.4±0.1	1.7±0.3	0.8±0.1	0.5 ±0.1	0.2±0.1	0.27
Testes	0.4±0.1	0.6±0.1	0.5±0.2	1.0 ±0.2	0.2±0.2	0.4±0.3
Brain	1.7±0.5	2.4±0.3	0.8±0.1	1.3 ±0.3	0.2±0.3	2.7±2.7
Lungs	6.7±0.5	8.2±0.7	2.4±0.9	3.1 ±1.5	0.4±0.2	0.8±0.4

Heart	2.1±1.3	3.4±2.2	0.6±0.3	
0.8 ±0.4	0.2±0.1	0.3±0.1		

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[a] Mean ± SD for three animals.

[b] From: Decad et al. (1981b).

ND = below limit for accurate detection.

Ioannou et al. (1983) calculated a whole-body half-life of approximately 40 days for a non-toxic dose of TCDF in young male Hartley guinea-pigs. Their estimation was based on the distribution of TCDF-derived radioactivity in liver, adipose tissue, skin, and muscle in three animals 36 days after a single oral dose of 4 mg TCDF/kg body weight and on certain approximations obtained from a previous study (Decad et al., 1981b). Failure to demonstrate a correlation between degree of bioaccumulation and lethality of TCDF in this study may be due partly to the calculations of body burden based on the uncertain estimate of the 40 days half-life for TCDF, which may not be valid for both toxic and non-toxic doses of TCDF.

9.1.2 Studies with other PCDFs

Young male Wistar rats absorbed approximately 68% of a single oral dose of 1.0 mg 2,3,4,7,8-pentaCDF/kg body weight given in salad oil (Yoshimura et al., 1986). The daily faecal excretion was about 0.1% of the administered dose/day, whereas no 2,3,4,7,8-pentaCDF was detected in urine. Four weeks after dosing the retention of 2,3,4,7,8-pentaCDF in the liver was 48.8% of the dose. The addition of 5% of activated charcoal beads to the diet, one week after dosing and throughout the study, increased the faecal elimination of 2,3,4,7,8-penta CDF about 3-fold, but had no effect on urinary elimination. Both the liver and extrahepatic tissues, except the kidney, from rats on basal diet supplemented with activated charcoal beads had lower levels of 2,3,4,7,8-pentaCDF than rats on basal diet only.

Yoshihara et al. (1981) administered single ip injections of 13 individual PCDF congeners (at 1, 5, or 10 µg/kg) to young male Wistar rats, and retention of the respective isomers in the liver was determined 5 days later. The great variation observed in the hepatic accumulation of the various isomers seemed to depend on the position as well as the number of chlorine atoms substituted. Isomers having vicinal hydrogens were accumulated to a lesser degree, although three

of the six isomers having no vicinal hydrogens (1,3,6,8-tetraCDF, TCDF, and 1,2,4,6,8-pentaCDF) also showed low accumulation. The isomer most highly accumulated was 2,3,4,7,8-pentaCDF, more than 65% of the dose being retained, whereas only 3.8% of TCDF, which is equally potent biologically, was retained. These results would imply that there is no relationship between hepatic distribution of PCDFs and their potential for acute toxicity. All animals in this study showed toxic symptoms and liver microsomal AHH activity was strongly induced, except in the cases of the following isomers: 2,8-diCDF, 1,2,7,8-tetraCDF, 1,3,6,7-tetraCDF, 1,3,6,8-tetraCDF, 1,2,4,6,8-pentaCDF. It is important to take this into consideration when judging the kinetic data. A mixture of 14% 1,2,7,8-tetraCDF, 35% TCDF, 1% 1,2,4,7,8-pentaCDF, 49% 1,2,3,7,8-pentaCDF, 1% 2,3,4,7,8-pentaCDF, and 1% hexaCDF was administered as a single ip

dose of 10 mg PCDF/kg body weight to young male Wistar rats (Kuroki et al., 1980). The retention of the isomers in the liver, 5 days post-treatment, showed good agreement with the results of Yoshihara et al. (1981).

Based on the purification of three isoenzymes of cytochrome P-450 and the recovery of ^{14}C-radioactivity from the hepatic microsomes of Wistar rats treated with ^{14}C-2,3,4,7,8-pentaCDF (single ip dose of 1 mg/kg body weight, 5 days previously), Kuroki et al. (1986) suggested that one of these isoenzymes, P-448 H, functions as the storage site of 2,3,4,7,8-pentaCDF in the rat liver.

Fly ash and crude or purified toluene extracts of PCDD- and PCDF-containing fly ash from a municipal incinerator (Zaanstad, The Netherlands) were mixed with ordinary laboratory diet for rats (van den Berg et al., 1983). Small portions (2 g) of these diets were fed to male Wistar rats (300 g) every 24 h for 19 days, at which time the animals were sacrificed. The levels of tetra-. penta-, and hexa-chlorinated PCDDs and PCDFs in samples of liver and adipose tissue from these rats were determined. Rats fed the fly ash-containing diet stored PCDDs and PCDFs in their livers at concentrations which were at least 3 to 5 times lower than those of rats fed with comparable amounts of fly ash extracts. For the pentaCDD, hexaCDF, and hexaCDD isomers these concentrations were approximately 10-20 times lower. Generally PCDFs had a higher retention in the liver of rats than the corresponding PCDDs. In the adipose tissue of rats fed with fly ash extracts, retention was higher for penta- and hexaCDDs than for the corresponding PCDFs.

In a later study, male Wistar rats (275 g) were fed a diet containing the same fly ash, pretreated with 2.5% HCl (van den Berg et

al., 1986a). A control group received standard diet. All congeners retained in the livers of the rats had a 2,3,7,8-chlorine substitution pattern. With the exception of 2,3,4,7,8-pentaCDF and 2,3,4,6,7,8-hexaCDF, the retention for each congener was below 10% of the dose. The retention percentages of the various congeners in the liver were almost equal at all time-points studied (34, 59, and 99 days), thus indicating a long half-life of these congeners in the liver of the rat.

A mixture of two tetraCDFs, four pentaCDFs, and four hexaCDFs, was given as a single ip injection of 500 µg to male ICR mice (Morita & Oishi, 1977). The distribution patterns of the isomers in various tissues were followed for up to 8 weeks. Analyses were performed with GC (with electron capture detection) and isomers were identified by peak number only. PCDFs were mainly located in the liver, spleen, and fat tissues, but low to minimal amounts were found also in the kidney, testes, lungs, heart, and brain. The GC patterns of liver samples changed markedly with time, in contrast to those of the other tissues, including fat, where the GC patterns remained similar throughout the

study. Most isomers with shorter retention times were readily absorbed and then rapidly disappeared from the liver. Isomers with longer retention times were slowly absorbed, and thus appeared later and persisted longer in the liver. If the mixture had been administered orally, those isomers with long retention times might have passed the gastrointestinal tract with very low absorption.

The hepatic retention of PCDDs and PCDFs after dietary intake of the above-mentioned HCl-pretreated fly ash was studied in male Golden Syrian hamsters (van den Berg et al., 1986b). The livers were analyzed for tetra-, penta-, and hexaCDDs and -CDFs after feeding the diet, which contained 25% fly ash. No detectable hepatic retention was observed after 34 days. The highest retention after 95 days was 8.4% for 2,3,4,7,8-pentaCDF, but the retention was generally below 5% of the total dose. With the exception of 2,3,4,6,7- pentaCDF, only 2,3,7,8-substituted PCDDs and PCDFs were retained. Constant relative concentrations were found for the 2,3,7,8-substituted PCDDs and PCDFs at the time-points studied.

In studies by Firestone et al. (1979), three lactating Holstein cows received commercial grade pentachlorophenol orally by gelatine capsule at a dose rate of 10 mg/kg body weight twice daily for 10 days and once daily for the following 60 days. One cow served as a control and received gelatine capsules containing only ground corn. The pentachlorophenol composite used contained ten PCDD congeners (0.1-690 mg/kg) and eight PCDF congeners (0.9-130 mg/kg). Faeces collected

on day 28 of the treatment period contained three hexaCDDs (0.05-0.63 µg/kg), two heptaCDDs (21.3-33.1 µg/kg), and octaCDD (290-429 µg/kg). Faeces also contained hexa-, hepta-, and octaCDF. Milk, body fat, and blood contained only three of the PCDD congeners present in the pentachlorophenol composite, namely 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD. Milk samples also contained hexa-, hepta-, and octaCDF. The average concentrations of 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, octaCDD, and octaCDF in the composite milk fat at the end of the treatment period were 20, 40, 25, and 2 mg/kg, respectively. Similar concentrations were found in body (shoulder) fat at the end of the treatment period (13, 24, and 32 mg/kg, respectively, for 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD). Levels of dioxins in the blood were about 1000 times below the values in milk or body fat. The average daily excretion of 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD in the milk during days 40 to 70 of treatment was about 20, 40, and 23 mg (corresponding, respectively, to 33, 3, and 0.6% of the daily intake of PCDDs). One hundred days after cessation of treatment the average levels of 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD in shoulder fat and milk fat were 2.5, 6.6, 5.6 mg/kg and 4.3, 6.9, 3.0 mg/kg, respectively.

Table 69. Levels of PCDFs in the liver of dams and in fetuses and offspring after oral administration of PCDFs to mice for 18 days during pregnancy[f]

PCDF congener[a]	Total intake of PCDFs by dams killed on day 18 of pregnancy (µg)[d]	% of PCDF intake in: liver of dam (week)		Total intake of PCDFs by dams killed 2 weeks after delivery (µg)	% of PCDF intake in: liver of dam	offspring	fetus
		1	2				
tetraCDF[c]	1.4±0.06[d]	5.4	ND		ND	ND	1.6±0.10 ND ND

	tetraCDF[c]	8.1±0.32			ND
ND	9.0±0.57	ND	ND	ND	
	2,3,7,8-tetraCDF	11.4±9.46			5.5
0.007	12.5±0.79	0.03	0.05	T[e]	
	pentaCDF[c]	2.9±0.12			5.7
ND	3.1±0.20	4.0	0.10	0.27	
	pentaCDF[c]	13.2±0.53			3.9
0.004	14.5±0.91	0.1	0.03	T[e]	
	2,3,4,7,8-pentaCDF	4.9±0.20			14.5
ND	5.4±0.34	10.0	0.29	0.89	
	hexaCDF[c]	1.4±0.06			10.7
ND	1.6±0.10	6.4	0.28	0.76	
	Total	43.2±1.73			5.2
0.003	47.7±3.0	1.6	0.07	0.14	

[a]   Nine dams in group killed on day 18 of pregnancy.

[b]   Ten dams in group killed on day 14 after delivery.

[c]   Specific isomer not determined.

[d]   Mean ± SEM.

[e]   T = 0.01–0.1 µg/kg total congener.

[f]   From: Nagayama et al. (1980).

ND = not detected.

9.2 Metabolic Transformation

Thirteen chlorinated compounds were detected in bile collected
for 48 h from female Sprague Dawley rats given a single oral dose of
678 µg TCDF (79.4% pure)/kg body weight (Poiger et al., 1984). The
four major metabolites considered to originate from TCDF were
trichloromethoxy-dibenzofuran, two trichlorodimethoxy-dibenzofurans,
and tetrachloromethoxy-dibenzofuran. The remaining nine metabolites,
detected in minute amounts, originated most likely from contaminating
PCDFs (1% triCDF, 8.4% tetraCDFs, 11.2% pentaCDFs).

Metabolites of 2-monoCDF, 2,8-diCDF, 2,3,8-triCDF, and octaCDF
were determined in the urine, faeces, fat, and liver of male Wistar
rats given single oral doses of 250 mg/kg body weight of the
respective isomers (Veerkamp et al., 1981). Analyses were performed

with GC-MS. No metabolites in any samples were found in rats given
octaCDF. Monohydroxy and dihydroxy derivatives were obtained with all
other isomers, whereas sulfur-containing metabolites were detected
only with the monoCDF and diCDF. Metabolites from 2-monoCDF and 2,3,8-
triCDF were found in urine and faeces only, but with 2,8-diCDF
metabolites appeared also in the tissues.

9.3 Transfer Via Placenta and/or Milk

Nagayama et al. (1980) studied the transport of a mixture of
PCDFs into the placenta and milk in the mouse (Table 69). A diet
containing 0.6 mg PCDFs/kg (48% tetraCDFs, 49% pentaCDFs, and 3%
hexaCDFs) was given for 18 days after mating. Nine dams were killed on
day 18 of pregnancy and 10 on day 14 after delivery. After giving
birth, the mothers were fed a diet free of PCDFs. The placental
transport, calculated from the amount of PCDFs in the neonates, was
about 0.003% of the administered dose. The isomers that remained in
the tissues were TCDF and 2,3,4,7,8-pentaCDF. While the levels of
PCDFs in the mothers dropped from 5.2% to 1.6% of the total intake,
the whole-body levels in the sucklings increased from 0.003% at the
time of birth to 0.07% after one week and to 0.14% of the total intake
after 2 weeks. TCDF and 2,3,4,7,8- pentaCDF were the dominant species
in both the mothers and the pups. To study the transport through milk
only, the same PCDF-containing diet was given to pregnant rats for 14
days from day 18 after mating, including the lactation period
(Nagayama et al., 1980) (Table 70). After 14 days 5.1% of the total
intake was found in the liver of the mother. The offspring contained
0.3% and 1.2% of the dam's intake after 1 and 2 weeks, respectively.
The dominant isomers recovered in the offspring were TCDF,
2,3,4,7,8-pentaCDF, and one unidentified pentaCDF, i.e., the same
isomers found in the largest amounts in the mother's liver. The data
demonstrated that the amounts of PCDFs transferred through milk were
much larger than the amounts transferred across the placenta.

Table 70. Levels of PCDFs in the liver of mouse dams and in offspring after oral
          administration of PCDFs to dams for 14 days following delivery[e]

PCDF congener	Total intake of PCDF (µg)[c]	% of PCDF intake in:[a]		
		liver of dam	offspring (week)	
			1	2

tetraCDF[b]	2.3±0.13	6.9	ND	ND
tetraCDF[b]	12.9±0.71	ND	ND	ND
2,3,7,8-tetraCDF	17.9±3.10	5.6	0.5	1.4
pentaCDF[b]	4.4±0.24	6.7	T[d]	0.6
pentaCDF[b]	21.0±1.14	3.7	0.4	1.6
2,3,4,7,8-pentaCDF	7.7±0.42	13.8	0.4	2.2
hexaCDF[b]	2.3±0.13	7.9	T[d]	1.5
Total	68.3±3.75	5.1	0.3	1.2

[a]   Ten dams in the group.

[b]   Specific isomer not known.

[c]   Mean ± SEM.

[d]   T = 0.01-0.1 µg/kg total congener.

[e]   From: Nagayama et al. (1980).

ND = not detected.

Weber & Birnbaum (1985) studied the distribution and placental transfer of a single oral dose of 800 µg [14]C- TCDF (0.0485 µCi/µg)/kg body weight to pregnant C57Bl/6 mice on gestation day 11. Embryo mortality on gestation days 12 to 14 was in the range 7.9 to 17.8%. No detectable radioactivity was found in the embryos whereas about 0.01% of the radioactive dose was contained in the placenta. The hepatic radioactivity in the dams decreased rapidly from 30.0% of the dose on gestation day 12 to 12.1% of the dose on gestation day 14. The cumulative urinary and faecal excretion from gestation day 12 to 14 were 5.4 and 80.1% of the administered dose, respectively.

10.   EFFECTS OF PCDFs ON ANIMALS

10.1 Acute Toxicity

Single oral LD^{50} values for TCDF in three species are listed in Table 71.

10.1.1 Studies on rats

No histological changes associated with TCDF toxicity could be observed in rats at oral doses up to 1000 µg TCDF/kg body weight (Moore et al., 1976). These preliminary results, which also mentioned

that only mild toxicological changes occurred in rats at 6000 µg
TCDF/kg body weight, have not been presented in a final report.

Intravenous administration of 30.6 µg TCDF/kg body weight to male
Fisher rats has been shown to cause listlessness, excessive hair loss,
and decreased weight gain 2 days post-treatment. These adverse effects
were reversible and 3 weeks after dosing the animals appeared healthy
with normal body weight. There were no signs of thymic or splenic
atrophy or of liver hypertrophy (Birnbaum et al., 1980).

Single ip injection of 1 or 10 mg/kg body weight of nine
individual PCDF isomers, with at least three chlorines in the lateral
positions, to male Wistar rats produced thymus atrophy and liver
hypertrophy 5 days post-treatment. Five other congeners having no more
than two chlorine atoms in the lateral positions did not cause any
effects on the thymus or liver within the same dose range (Yoshihara
et al., 1981).

The ability of 15 individual PCDFs to affect body weight gain and
thymic atrophy in immature male Wistar rats was investigated 14 days
after a single ip injection (Ganon et al., 1985). The ED^{50} values
for both effects were estimated for each congener (Table 61).

10.1.2 Studies on mice

Moore et al. (1976) failed to establish a lethal dose for TCDF in
C57Bl/6 mice after giving single oral doses of up to 6000 µg/kg body
weight with an observation period of 30 days. However, there was a
transient depression in body weight gain, thymic involution, and mild
hepatotoxicity when 6000 µg TCDF/kg was given subcutaneously. Poland
& Glover (1980) found TCDF-induced thymus atrophy in C57Bl/6 mice 5
days after a single ip dose of 3×10^{-7} mol/kg body weight.

Single doses of 100 to 1000 µg TCDF/kg body weight to pregnant
C57Bl/6 mice on gestation days 10 to 13 produced no toxic effects on
the dams within the time studied (Hassoun et al., 1984a; Weber et al.,
1984).

A mixture of two tetraCDFs, four pentaCDFs and four hexaCDFs
given as a single ip dose of 500 mg to ICR mice produced no
deathswithin 8 weeks (Morita & Oishi, 1977). CF-1 mice given a PCDF
mixture containing 42% tetraCDFs, 54% pentaCDFs, and 4% hexaCDFs as a
single oral (10 to 1000 mg/kg body weight), sc (10 to 200 mg/kg), or
ip (10 to 100 mg/kg) dose developed no toxic signs during the first
week, although a modest weight loss was noted (Nishizumi, 1978). The
first deaths occurred 8 days after an oral dose of 1000 mg/kg, 5 weeks

after a sc dose of 200 mg/kg, and 11 days after an ip dose of 100 mg/kg. The oral LD_{50} (30 day) was 184 mg/kg for males and 414 mg/kg for females. Hepatomegaly and thymus atrophy were consistent findings in mice that died. In surviving mice on high dosages, the liver exhibited small necrotic foci accompanied by cellular infiltrates. The hepatic lesions occurred in the centrilobular area, and enlarged hepatocytes containing foamy cytoplasm, increased numbers of lipid droplets, and proliferation of smooth endoplasmatic reticulum were also seen.

10.1.3 Studies on guinea-pigs

In studies by Moore et al. (1979), the patterns of toxicity were similar for TCDF, 2,3,4,7,8-pentaCDF, and 2,3,7,8-tetrabromodibenzofuran when given to young Hartley guinea-pigs. The single oral LD_{50} was 5-10 µg/kg body weight for all three isomers, and the time to death ranged from 8 to 26 days. Overt signs at lethal doses were immediate and progressive weight loss, rough soiled hair coat, listlessness, and dehydration. Similar symptoms appeared 3 days after an iv injection of 6 µg TCDF/kg body weight (Decad et al., 1981b). At necropsy lack of body fat and reduced body mass and thymus weight were found. Histological findings were primarily associated with the depletion of lymphoid cells in the thymic cortex, but hypocellularity of bone marrow and hyperplasia in epithelial cells of the renal pelvis, ureter, and urinary bladder were also observed. Liver lesions were not observed. Surviving animals showed mild thymic lymphoid hypoplasia only. Sublethal doses resulted in decreased body weight gain.

In a study by Ioannou et al. (1983), all three adult male Hartley guinea-pigs survived a single oral dose of 6 µg TCDF/kg body weight for at least 17 days. At 10 and 15 µg TCDF/kg body weight, deaths occurred on days 15-39 (two animals were sacrificed on day 17) and 13-20, respectively. The acute oral toxicity of soot (and of benzene extracts of the soot) containing PCDDs and PCDFs from a PCB-containing transformer fire (Binghamton, New York, USA) were studied in female Hartley guinea-pigs (Silkworth et al., 1982). As discussed in section 8.1.1.1, toxicities were noted at 100 and 500 mg/kg body weight of soot, but not when 1 and 10 mg/kg were administered.

Table 71. Single lethal dose values for TCDF[a]

Duration of study (days)	Species/strain LD_{50} (µg/kg body weight)	Sex/No	Time to death (days)	Age or weight	Dose tested (µg/kg[c] body weight)
	Mice				
	(C57Bl/6)	M/8		6 weeks	0
30	> 6000	not reported			400
					600
					800
					1200
					1500
					2500
					4000
					6000
	Guinea-pigs				
	(Hartley)	M/6		3-4 weeks	0
30	5-10	9-20			1
					5
					10
					15
	Monkeys				
	(Macaca	F/2		2.0-3.7 kg	0
60	1000	14-31			
	mulatta)				300
					1000
					1500

a From: Moore et al. (1979).
b M = male; F = female.
c Doses were given orally in corn oil.

10.1.4 Studies on rabbits

When the above-mentioned soot (or benzene extracts thereof) was

applied dermally to New Zealand white rabbits (Silkworth et al., 1982) (see section 7.4.4.1), it produced no overt toxicity, weight loss, or histological changes in thymus, kidney, or skin, but centrilobular hypertrophy was found in both sexes. The soot extract gave rise to a reversible skin inflammation and hepatic centrilobular hypertrophy in females only. Histological examination showed no changes in kidney, thymus, and skin.

10.1.5 Studies on monkeys

The single oral LD_{50} value for TCDF in the young female rhesus monkey (Macaca mulatta) was found to be 1000 µg/kg in a 60-days study within the dose range 0, 500, 1000, and 1500 µg/kg and with two or four animals at each dose level (Moore et al., 1979). The two monkeys that received the sublethal dose developed skin lesions and had decreased body weight gain. With lethal doses the following overt signs occurred after 7 to 10 days: progressive weight loss, loss of body fat, facial oedema, loss of facial hair, loss of finger and toe nails, and thickening of skin. Death occurred within 2 to 4 weeks. Major histological findings included hyperkeratosis of the skin, thymic atrophy with lymphoid hypoplasia, and adverse effects on epithelial linings. No structural liver lesions were observed though the liver weight was increased. Increases in serum albumin and cholesterol were also recorded.

Three male rhesus monkeys given a single dose of 30.6 µg TCDF/kg body weight did not gain weight during the three following weeks, and they developed facial skin lesions, mainly of sebaceous glands (Birnbaum et al., 1981).

10.2 Short-Term Toxicity

10.2.1 Studies on rats

Male Sprague Dawley rats were fed 1 or 10 µg/kg of a PCDF mixture, containing two tetraCDFs, four pentaCDFs, and four hexaCDFs for 4 weeks (Oishi et al., 1978). Both diets gave rise to decreases in growth rate, food consumption, haemoglobin and haematocrit values, erythrocyte counts, serum levels of triglyceride, testosterone, glutamic pyruvic transaminase, and leucine aminopeptidase activities, as well as increases in serum cholesterol, cholinesterase, and glutamic oxaloacetic transaminase activities. Rats fed the 10 µg/kg diet developed chloracne-like lesions on the ears within 3 weeks. Furthermore, this diet decreased the relative weights of thymus, prostate, and seminal vesicles and increased the relative weights of liver, testes, spleen, adrenals, lung, heart, and brain. In another

similar study, no effects were seen on total serum proteins or
leukocyte counts (Oishi, 1977).

In studies by Hori et al. (1986), male Sprague Dawley rats (aged
5 weeks) were for 21 days given daily oral doses of a mixture of PCBs,
PCQs, and PCDFs having a similar composition and isomeric ratio to
those found in the contaminated rice oil causing "Yusho" (section
11.1). The toxicities noted included thymic atrophy, suppression of
weight gain, hepatic enlargement, and an increased serum cholesterol
level and a decrease in serum glutamic pyruvic transaminase activity.
The mixture caused an induction of the AAH drug-metabolizing enzyme
similar to that caused by PCDFs alone. These results support the
hypothesis that the predominant etiology of "Yusho" involves PCDFs
contained in the PCB-contaminated toxic rice oil.

10.2.2 Studies on mice

Mice (C57Bl/6), given TCDF orally 5 times per week for 30 days
did not develop clinical signs of toxicity at doses of 30, 100, or 300
µg/kg body weight. However, thymus atrophy, liver hypertrophy,
decreased leukocyte count, and slightly elevated total serum protein
did occur in the high dose group at the end of the study (Moore et
al., 1979). Daily doses of 10, 30, or 50 µg TCDF/kg body weight on
gestation days 10-13 produced a dose-related increase in maternal
liver weight in C57Bl/6 mice (Weber et al., 1984). Decreased thymus
weight was recorded in ICR/JCL mice exposed to four weekly doses of a
mixture (at 100 µg/kg) of 12% tetraCDF and 88% pentaCDF (Oishi &
Hiraga, 1980). When PCDFs of unknown composition were given in the
diet at 0.6 mg/kg to mice for 10 weeks, severe dermal lesions,
hyperkeratosis, and dilated hair follicles filled with keratinous
material occurred in 7 of 12 mice. Furthermore, hepatocytes had
enlarged nuclei and vacuolations in the cytoplasm (Nagayama et al.,
1979). Feeding female ddN mice 0.6 mg PCDFs/kg diet (48% tetraCDFs,
49% pentaCDFs, and 3% hexaCDFs) for 18 days after mating, or for 14
days after delivery, produced no overt toxic effects in dams or in
offspring (Nagayama et al., 1980).

10.2.3 Studies on guinea-pigs

In studies by Luster et al. (1979b), oral administration of 0.05,
0.17, 0.5, or 1.0 µg 2,3,7,8-TCDF/kg body weight once weekly for 6
weeks to young female Hartley guinea-pigs produced 30% mortality in
the high-dose group. The thymus weight was decreased in the 0.5 and
1.0 µg/kg dose groups, though histologically only a slight decrease in
the density of thymic cortex was observable. Reduction in spleen
weight or alterations in splenic morphology did not occur, neither was

there a consistent decrease in body weight.

Four adult male Hartley guinea-pigs were given six or seven weekly doses of 1 µg TCDF/kg body weight (Decad et al., 1981a). The animals started to lose weight rapidly after the fifth or sixth dose, the cumulative dose then being comparable to the oral LD_{50} value for young guinea-pigs. At this time all animals were moribund and by day 44 the first animal died. Neither hepatomegaly nor thymic atrophy was observed in this study. Thus multiple sublethal doses of TCDF appear to have a cumulative effect, and may lead to a critical body burden that will result in irreversible and progressive weight loss eventually followed by death.

Weekly oral doses of 1 µg TCDF/kg body weight or biweekly doses of 2 µg TCDF/kg body weight (interrupted by a 4-week period of no dosing after the fourth dose) to young male Hartley guinea-pigs in groups of four resulted in deaths on days 47, 51, 84; 31, 38, 88; and 32, 70, 85, respectively (Ioannou et al., 1983). At each dosing schedule one animal was sacrificed at 101 days after exposure. Repeated small doses, with various intervals in between, resulted in a similar lethality but a less dramatic weight loss than with a high acute dose.

10.2.4 Studies on rabbits

The 25% ether-hexane extracts from two commercial polychlorinated biphenyl (PCB) preparations containing tetraCDFs and pentaCDFs produced hyperplasia and hyperkeratosis of the follicular epithelium of the rabbit ear skin when applied dermally weekly for 3 weeks in a dose corresponding to 200 mg PCB. Liver lesions or decreased weight gain were not observed. No dermal effects could be found when an ether-hexane extract from a PCB preparation lacking PCDF impurities was applied in the same manner (Vos & Beems, 1971).

A mixture of tetraCDFs and pentaCDFs was much less potent than was TCDD in producing hyperkeratosis when applied to the inside of depilated rabbit ears for 3 consecutive days (Nishizumi et al., 1975).

10.2.5 Studies on hamsters

No toxic effects were reported in male Golden Syrian hamsters (50-70 g) given a diet containing 2.5% HCl-pretreated fly ash from a municipal incinerator (Zaanstad, The Netherlands) for up to 95 days (van den Berg et al., 1986b).

10.2.6   Studies on monkeys

     A two-month study with three young male rhesus monkeys (Macaca
mulatta), serving as their own controls and fed a diet with 50 µg
TCDF/kg, resulted in one case of illness after 1 month and one death
after 2 months when the cumulative dose was calculated to be 300 µg/kg
(McNulty et al., 1981, 1982a). Toxic changes observed after 1 month
included periorbital oedema, reddening and thickening of the eyelids,
enlargement of facial hair follicles, and decreased number and size of
sebaceous glands in the skin. After 2 months these changes had become
more severe and were accompanied by decreased physical activity and
elevated (or eventual loss of) toe and finger nails. There were no
changes in haematology or serum chemical values. The diseased and the
surviving monkeys recovered rapidly when they were returned to

uncontaminated food. Within 3 months, behaviour, clinical appearance,
and histological structure of the skin were normal. The monkey that
died had lost 23% of its initial weight and most of its body hair.
Sebaceous glands were replaced by small squamous cysts. Severe lesions
were confined to the skin, thymus, and the stomach epithelium, whereas
liver lesions were modest. Decreased bone marrow cellularity was a
postmortem finding which was not reflected in the peripheral blood
count taken before death.

10.2.7   Studies on chickens

     Mortality in one-day-old White Leghorn chickens given 1 or 5 µg
TCDF/kg body weight orally for 3 weeks was 16% and 100%, respectively,
with an average time to death of 19 and 11.5 days (McKinney et al.,
1976). Body weight gain and food consumption were decreased during the
third week post-treatment. Dose-related subcutaneous oedema, ascites,
and hydropericardium, as well as thymus atrophy, occurred. Depletion
of lymphatic cells was evident both in the spleen and thymus. Mild
liver lesions were found only in the high-dose group. Total serum
protein and serum albumin were reduced.

     The significant difference in toxicity in chickens between three
commercial PCB preparations (Vos & Koeman, 1970) was later
demonstrated to be caused by the presence of tetra- and pentaCDFs in
two of the three preparations (Vos et al., 1970). The 25% ether-hexane
extracts from these two PCB preparations were highly toxic in the
chick embryo assay (Vos et al., 1970), whereas no effect could be
produced by the extract from the PCB preparation lacking PCDF
impurities.

10.3   Chronic Toxicity

10.3.1 Studies on monkeys

        In studies by McNulty et al. (1981, 1982a), three young male
rhesus monkeys (<u>Macaca mulatta</u>) were exposed for 6 months to 5 µg
TCDF/kg diet, and one animal served as a control. One animal was
killed after 6 weeks when moribund. Overt toxic signs in the two
remaining animals started to appear after 3 months and the symptoms
remained for the following 3 months. One of these animals died
suddenly after 6 months. The remaining monkey was returned to normal
food and rapidly recovered. Clinically and pathologically, chronic
intake of small amounts of TCDF caused symptoms similar to those
following a single large dose of TCDF (section 9.1.2.4) or acute or
chronic ingestion of TCDD (see sections 7.1.1 and 7.3). The major
histopathological changes in all cases were seen in the thymus,
sebaceous glands, nail beds, bone marrow, and mucosa of the stomach
and bile ducts. The toxic potency of TCDF when ingested chronically
was approximately equal to that of TCDD. This contrasts with the acute
toxic effect of TCDF, which is approximately 20 times less than that
of its TCDD counterpart. The reason for death in the TCDF-poisoned

monkeys was obscure; it was preceded by weight loss, anorexia, and
depression. Only modest thymic and epithelial changes were present,
and there was no evidence for liver damage. The quick recovery of
animals returned to normal diet contrasted with the course of TCDD
poisoning in which illness progressed to death, or recovery was much
delayed, even after exposure had ended.

10.4 Effects Detected by Special Studies

10.4.1 Immunobiological effects

        To date no studies have been performed on the effects of PCDFs on
the developing immune system.

        Comparative studies on humoral immune responses in mice have
revealed that TCDF produces a pattern of responses similar to that
found for TCDD but only at 30-fold higher doses. Furthermore the
immunosuppressive effect of TCDD is much more persistent (Vecchi et
al., 1983b).

10.4.1.1 Histopathology

        During toxicity studies with pure isomers of PCDFs or with
mixtures of PCDFs, thymus atrophy has been noted as a consistent
effect in the mouse (Nishizumi et al., 1978; Moore et al., 1979), rat

(Oishi, 1977; Oishi et al., 1978), guinea-pig (Moore et al., 1979),
and monkey (Moore et al., 1979; McNulty et al., 1981). Studies aimed
at investigating immunobiological effects revealed decreased thymic
weights (Luster et al., 1979b; Vecchi et al., 1983). The histological
findings are similar to those occurring after TCDD exposure, i.e.,
loss of lymphoid cells in the thymic cortex. A reduced number of
spleen cells was obtained from mice treated with a single ip dose of
180 µg TCDF/kg body weight (Vecchi et al., 1983), but no splenic
pathology was reported in mice given four weekly oral doses of a PCDF
mixture (at 100 µg/kg) containing 12% tetraCDFs and 88% pentaCDFs
(Oishi & Hiraga, 1980). Peritoneal cell and macrophage counts in mice
were not modified by an ip dose of TCDF (180 µg/kg body weight)
(Vecchi et al., 1983).

10.4.1.2 Humoral-mediated immunity

     Adult female Hartley guinea-pigs exposed orally to 0.05, 0.17,
and 0.5 µg TCDF/kg body weight once weekly for 6 weeks showed somewhat
depressed serum IgG concentrations. A dose-related depression in
splenic lymphocyte proliferation was seen in TCDF-treated animals
after stimulation with the B-lymphocyte mitogen Escherichia coli
0127 lipopolysaccharide at 50 µg/ml medium. There were no effects on
any of the major serum proteins, neither was there an effect on the
antibody response towards bovine gamma globulin (BGG) (Luster et al.,
1979b).

     The antibody response to sheep red blood cells given 7 days after
a single ip injection of 180 µg TCDF/kg body weight was inhibited by
85% and 35% in C57Bl/6 and DBA/2 mice, respectively (Vecchi et al.,
1983), whereas a single ip dose of 10 µg TCDF/kg body weight to
C57Bl/6 mice had no effect (Rizzardini et al., 1983). The suppression
noted by Vecchi et al. (1983) was dose dependent as well as time
dependent; by day 42 post-treatment a near-normal antibody response
was obtained.

10.4.1.3 Cell-mediated immunity

     Oral intubation of 10 or 100 mg PCDF (12% tetraCDFs and 88%
pentaCDFs) per kg body weight once weekly for four weeks increased the
sensitivity to endotoxin of ICR/JCL mice. Following an ip injection of
50, 250, or 500 µg endotoxin per mouse a dose-dependent increased
mortality was noted two days after the final treatment with PCDF
(Oishi & Hiraga, 1980). Only at high dose levels were there any
effects on cell-mediated immunity functions in female Hartley
guinea-pigs given 0.05, 0.17, 0.5, or 1.0 mg TCDF/kg body weight
orally once weekly for six weeks (Luster et al., 1979b).

Both the depression in delayed hypersensitivity response to purified protein derivative and the ability of BGG-sensitized lymphocytes to release the macrophage inhibition factor were related to the dose of TCDF. Splenic lymphocytes from TCDF-treated animals, stimulated with the T-lymphocyte mitogen phyto-haemagglutinin (PHA), showed a decreased proliferation. On the other hand proliferation of splenic lymphocytes stimulated with concanavalin A (Con A), another T-lymphocyte mitogen, showed no TCDF-related effect. The increased proliferative response to Con A and PHA in thymocytes co-cultivated with thymus epithelial (TE) cells or cultivated in TE-conditioned medium was inhibited if the TE cells were pretreated with TCDF for 48 h, thus suggesting a direct effect on TE cells (Osborne et al., 1984).

10.4.2 Enzyme induction

Studies discussed below show that PCDFs are potent enzyme inducers, the enzyme-inducing potencies varying greatly depending on the position as well as on the number of chlorine atoms substituted. The structure-activity relationships of the PCDFs with regard to enzyme induction are similar to those for PCDDs, with TCDF and 2,3,4,7,8-pentaCDF being the most potent (Tables 56 and 61).

10.4.2.1 Studies on rats

Intraperitoneal doses of 2.5 mg TCDF/kg body weight given once daily for three days to female CD rats induced 38- and 3-fold increases, respectively, in AHH and UDPGT activities 24 h after the final dose. The cytochrome P-450 content was doubled but no effect was found on the aminopyrine $\underline{N}$-demethylase activity (Goldstein et al., 1978).

Increased AHH and EROD activities were found in the hepatic microsomal fraction from immature male Wistar rats 5 days after ip injection of single doses of TCDF (1.7 µmol/kg body weight) or 2,3,4,7,8-pentaCDF (0.3, 1.5, 3.0 µmol/kg body weight) (Keys et al., 1985). This study also detected an alteration by TCDF in the hepatic metabolism of testosterone in these rats. Yoshihara et al. (1981) gave a single ip dose of 1, 5, or 10 mg PCDF/kg body weight of 13 individual PCDFs to young male Wistar rats five days prior to the determination of hepatic enzyme activities. Congeners having at least three chlorine atoms in the lateral positions typically showed increased AHH and DT-diaphorase activities, while those congeners having no more than two chlorine atoms in these positions were not inductive. The cytochrome P-448 content was increased by 5 of the 13 congeners whereas the benz-phetamine-$\underline{N}$-demethylase activity was

depressed by 7 of the 13. The most potent isomers, TCDF and 2,3,4,7,8-pentaCDF, were effective at a single dose of 1 µg/kg body weight. The ranking of the potency for enzyme-inducing abilities did not coincide with the hepatic distribution of the test substances. Hepatic AHH activity in male Wistar rats was significantly enhanced only by TCDF and 2,3,4,7,8-pentaCDF among the 15 individual PCDF isomers tested, the dose administered intraperitoneally being 5 µg PCDF/kg body weight (Nagayama et al., 1983).

Eight of the 15 PCDF isomers tested increased the pulmonary AHH activity from 5-fold to 30-fold. In this study no PCDF-related AHH induction was present in the kidney, prostate, thymus, or spleen. Bandiera et al. (1984b) investigated the effect of three tetraCDFs and three pentaCDFs at doses of 500 and 1000 µg/kg body weight, respectively, on hepatic AHH, aminopyrine N-demethylase, 4-chlorobiphenyl hydroxylase, and EROD activities in male Wistar rats. The most active compounds, TCDF and 2,3,4,7,8-pentaCDF, were potent inducers of the cytochrome P-448-dependent monooxygenases. Some induction of microsomal AHH, EROD, and 4-chlorobiphenyl hydroxylase was observed also for the TCDF and 1,2,4,7,9-pentaCDF.

The ED_{50} values for hepatic AHH (Table 61) and 4-chlorobiphenyl hydroxylase induction were established for 15 individual PCDFs in immature male Wistar rats 14 days after a single ip injection (Mason et al., 1985).

Significant induction of hepatic AHH activity in male Sprague Dawley rats was given only by 3 out of 25 individual PCDFs given as single oral doses of 40 µg/kg body weight (Doyle & Fries, 1986). The active congeners were 2,7-diCDF, TCDF, and 2,3,4,7,8-pentaCDF.

A mixture of PCDFs, reconstituting the approximate composition found in the liver of Yusho victims (7.4% tetraCDF, 6.1% 1,2,4,7,8-pentaCDF, 19.0% 1,2,3,7,8-pentaCDF, 29.4% 2,3,4,7,8-pentaCDF and 39.1% 1,2,3,4,7,8-hexaCDF by weight) was given as a single ip injection to male Wistar rats 14 days before measuring the induction

of cytochrome P-448-related enzyme activities (Bandiera et al., 1984a). A dose-related enhancement of AHH and EROD activities was found within the range 10 to 400 µg PCDF mixture/kg body weight.

10.4.2.2 Studies on mice

No induction of cytochrome P-448 content, or of ECOD activities, was found 12 days after a single ip injection of 10 µg TCDF/kg body weight to male C57Bl/6J mice (Rizzardini et al., 1983).

Nagayama et al. (1985) investigated the AHH-inducing potency of TCDF, 2,3,6,7-tetraCDF, 1,2,3,6,7-pentaCDF, 1,2,3,7,8-pentaCDF, 2,3,4,6,7-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,6,7-hexaCDF, and 1,2,3,4,7,8-hexaCDF in two strains of responsive (C57Bl/6 and AKR/Qdj) and two strains of non-responsive (DBA/2 and DDD) mice. All congeners were given as single ip doses of 30 µg/kg body weight in olive oil. No single congener induced the AHH activity above the control level in the non-responsive mice. Significantly increased AHH activity was found in both responsive strains exposed to TCDF, 1,2,3,7,8-pentaCDF, and 2,3,4,7,8-pentaCDF. Mice (C57BL/6) treated with 2,3,4,6,7-pentaCDF and 1,2,3,4,7,8-hexaCDF also responded with increased AHH activity.

10.4.2.3 Studies on chickens

Hepatic AHH activity in chick embryos was inducible by TCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,7,8-pentaCDF, with ED_{50} values of 0.015, 0.014, and 0.071 nmol/egg, respectively (Poland et al., 1976). No induction was produced by unchlorinated dibenzofuran, 2,8-diCDF, 2,4-diCDF, 2,4,8-triCDF or 1,3,6,7-tetraCDF at the doses tested. There were no effects on ALA synthetase, p-nitrophenol-UDPGT and testosterone-UDPGT activities. However, a modest increase in cytochrome P-450 content was present in one-day-old White Leghorn chickens 3 weeks after treatment with a single oral dose of 1 µg TCDF (Goldstein et al., 1976).

10.4.2.4 Studies on cell cultures

Exposure of primary hepatocytes isolated from adult male Wistar rats to TCDF for 72 h resulted in a 2-fold increase in AHH induction at 10^{-9} mol/litre and half-maximal induction at 3×10^{-10} mol/litre. However, no AHH induction was observed with 2,7-diCDF in the range 10^{-11} to 10^{-8} mol/litre in the same system (Jansing & Shain, 1985). A 59-fold increase in AHH activity and a 40-fold increase in EROD activity were obtained in rat hepatoma H-4-II E cells when exposed to 5×10^{-10} mol TCDF/litre for 3 days (Keys et al., 1986). In this same study it was demonstrated that TCDF had an additive effect, whereas 1,3,6,8-tetraCDF and 2,4,6,8-tetraCDF had counteracting effects on TCDD-induced enzyme induction.

The EC_{50} values for AHH and EROD induction (Table 61) have been established for 35 individual PCDFs in the rat hepatoma H-4-II E cell line (Bandiera et al., 1984b; Mason et al., 1985). AHH and EROD activities were determined after exposing the cells to optimal doses of PCDFs for 5 days. Unchlorinated dibenzofuran, or 2- and

3-chlorodibenzofuran did not induce these enzyme activities. EC50 values for all the remaining congeners varied between 10-4 and 1.3 x 10^{-10} mol/litre, the most active inducer being 2,3,4,7,8-PCDF.

Human lymphoblastoid cell lines, derived from the peripheral blood of healthy male and female volunteers of various ages, were exposed to eight individual PCDF isomers for 48 h (Nagayama et al., 1985b). The AHH inducibility was highly variable between individuals but less variable between isomers. In this system TCDF was about half as potent as 2,3,4,7,8-pentaCDF, 1,2,3,4,6,7-hexaCDF, or 1,2,3,4,7,8-hexaCDF, which were equally as potent as TCDD in inducing AHH.

A mixture of PCDFs, reconstituted on the basis of PCDF residues in the liver samples from Yusho victims (see section 10.4.2.1), had EC_{50} values for induction of AHH and EROD activities of 1.02 x 10_{-10} and 3.23 x 10_{-10} mol/litre, respectively, in the rat hepatoma H-4-II E assay (Sawyer & Safe, 1985). The calculated EC_{50} values based on the relative isomer content of the mixture were 3.07 x 10^{-10} and 4.43 x 10^{-10} mol/litre, respectively.

10.4.3 Receptor binding

The competitive binding of PCDFs to the TCDD receptor protein has been studied in vitro both in the hepatic cytosol (Poland et al., 1976; Bandiera et al., 1984b) and in the nucleus (Poellinger et al., 1982). Poland et al. (1974) investigated the ability of seven PCDF congeners to compete with TCDD in binding to the hepatic cytosol receptor from C57Bl/6J mice. They found the relative binding affinities for TCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,7,8-pentaCDF to be 37%, 34%, and 38%, respectively, of the binding affinity between TCDD and the receptor. The EC_{50} values for the competitive binding of 33 individual PCDFs to the receptor from rat hepatoma H-4-II E cell cultures varied from less than 10^{-3} mol/litre for 4-chlorodibenzofuran to 1.5 x 10^{-8} mol/litre for the most active competitor, 2,3,4,7,8-pentaCDF, which had an EC_{50} value comparable to that for TCDD, i.e., 1.0 x 10^{-8} mol/litre (Table 61) (Bandiera et al., 1984b; Mason et al., 1985). Of the TCDD bound to the nuclear receptor in vitro, 58% was displaced by a 100-fold molar excess of TCDF. These nuclei were isolated from the liver of Sprague Dawley rats pretreated intravenously with 1 µg TCDF 2 h prior to the incubation (Poellinger et al., 1982).

10.5 Embryotoxicity and Reproductive Effects

TCDF has been found to be a potent teratogen in mice at doses that produce no overt toxic effects in dams. Malformations observed include cleft palate and kidney malformation similar to hydronephrosis. Dose-related increases in fetal mortality occur with single high doses. The teratogenic pattern of TCDF thus is strikingly similar to that of TCDD (see section 7.5).

Single doses of 100 to 1000 µg TCDF/kg body weight to pregnant C57Bl/6 mice on gestation days 10 to 13 produced dose-related increases in the number of cleft palates and kidney malformations; both the number of litters and the number of fetuses were affected (Hassoun et al., 1984a; Weber et al., 1984). No other treatment-related malformations were reported. A cleft palate incidence of 40% was obtained in NMRI mice offspring after sc treatment of the dams on gestation days 9 to 11 with 200 nmol TCDF/kg body weight (Krowke, 1986).

Palatal closure in mice occurs late on day 14 of gestation, and so it is somewhat peculiar that the peak sensitivity for cleft palate occurs on day 12 (Hassoun et al., 1984a). The peak sensitivity for kidney malformation in mice occurs on day 11 of gestation (Hassoun et al., 1984a). The quantitative data on this malformation somewhat conflict in the two studies. Weber et al. (1984) reported that 95.5% of the fetuses had kidney malformations after a dose of 500 µg/kg body weight on day 10 of gestation. However, only 17% of the fetuses per dam had this malformation after a single dose of 400 µg/kg body weight on the same day in the study of Hassoun et al. (1984a). The difference might be due to unequal judging of the malformation. Preliminary results (Weber et al., 1984), suggested that TCDF-induced kidney malformations, up to a certain degree, represent a reversible defect since no hydronephrotic kidneys were found in neonates, whereas in identically treated dams examined on day 18 of gestation over 80% of the fetuses/litter were affected. Fetal mortality increased in a dose-related manner with high single doses administered on days 10 to 12. Peak sensitivity occurred on day 10 (Hassoun et al., 1984a). Multiple low dosing on gestation days 10 to 13 was more effective in producing fetal malformations, but less effective in producing fetal deaths, than single high dosing on day 10 (Weber et al., 1984). No effect on fetal mortality (days 12, 13, and 14) was observed in C57BL/6N mice given a single oral dose of 800 mg TCDF/kg body weight on day 11 of gestation (Weber & Birnbaum, 1985).

Recombinant inbred strains of C57Bl/6 and DBA/2 mice segregating at the Ah locus respond differently to the teratogenic effect of TCDF (Hassoun et al., 1984b). Fetuses of Ah-responsive strains responded

with a high frequency of cleft palates and kidney malformations after a single ip dose of 600 µg TCDF/kg body weight on day 12 of gestation.

However, no cleft palates and only modestly increased numbers of kidney malformations in a few strains were found with the same treatment in Ah-nonresponsive strains.

A diet containing 0.6 mg PCDFs/kg (48% tetraCDFs, 49% pentaCDFs, and 3% hexaCDFs), fed to mice for 18 days after mating, had no effect on the number or body weight gain of the offspring, neither were there any malformations related to the diet (Nagayama et al., 1980).

Three PCDFs, namely 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexa CDF, are teratogenic to C57BL/6N mice when administered orally by gavage on gestation days 10-13. A significant increase in hydronephrosis and cleft palate was found, with 2,3,4,7,8-PCDF being the most potent PCDF studied, having an ED_{50} of 36 µg/kg body weight for cleft palate and 7 µg/kg for hydronephrosis. For all three PCDFs, hydronephrosis occurred at a lower dose than did cleft palate (Birnbaum et al., 1987).

It has been pointed out by McNulty (1985) that chlorinated compounds such as 2,3,7,8-TCDD produce cystic periodontal lesions and squamous metaplasia of the ameloblasts surrounding unerupted teeth in rhesus monkeys. These findings are similar to those on the teeth development seen in Yusho patients (section 11.1).

10.6 Mutagenicity

When tested in Salmonella typhimurium strains KTA98 and TA100 with and without metabolic activation, no mutagenic activity was found for 2,9-diCDF, 3,6-diCDF, TCDF, or octaCDF (Schoeny, 1982). TCDF was also studied in Saccharomyces cerevisiae strain MP-1 and was found to be negative for forward mutation, mitotic crossing over, and mitotic gene conversion at concentrations up to 1000 mg/litre. Stationary phase cells were tested in the absence of exogenous activation (Fahrig et al., 1978).

10.7 Carcinogenicity

The hepatic tumour-promoting activity of a commercial polychlorinated biphenyl mixture, Aroclor 1254, with (Ar 1254) or without (Ar 1254-PCDF) PCDF impurities, was studied in Sprague Dawley rats pretreated with 66 µg diethylnitrosamine/ml drinking water for 5 weeks (Preston et al., 1981). Thereafter the rats were fed a diet supplemented with 100 µg Ar 1254/kg ($\geq$ 3 mg PCDF/kg) or Ar

1254-PCDF (<0.1 mg PCDF/kg) for 18 weeks. Examination of liver lesions by light microscopy demonstrated that Ar 1254 promotes formation of hepatocellular carcinomas in rats. The promoting incidence of 64% remained essentially unchanged when PCDF was removed from Ar 1254 by adsorption chromatography. Due to the high incidence of hepatocellular carcinomas produced by Ar 1254-PCDF itself, an additional effect of PCDF might have been difficult to measure in this study.

11.   EFFECTS OF PCDFs ON HUMAN BEINGS

Braun (1955) was the first to report chloracne due to chlorinated dibenzofurans, subsequently experimentally proven by Bauer et al. (1961). Vos et al. (1970) identified by mass spectrometry the presence of chlorinated dibenzofurans in commercial PCB mixtures, accounting for their acnegenic properties.

11.1 Yusho and Yu-cheng

A mass outbreak of food poisoning occurred in western Japan in 1968 following ingestion of a commercial brand of rice oil contaminated with polychlorinated biphenyls (PCBs) and related hydrocarbons. The poisoning was named "Yusho" (oil disease). Epidemiological proof of the cause of the epidemic depended on the demonstration of a dose-response relationship between the consumption of the toxic rice oil and the incidence of the poisoning or between the oil consumption and the clinical severity of the reaction. Approximately 2000 cases were recognized. In 1969, Japanese scientists first reported that the toxic rice oil which caused Yusho was contaminated with polychlorinated biphenyls (Tsukamoto et al., 1969). A few years later, the oil was found also to be contaminated with a smaller quantity of PCDFs (Nagayama et al., 1976) and a relatively large amount of polychlorinated quaterphenyls (PCQs) (Masuda & Yoshimura, 1982).

In March 1979, an epidemic of a peculiar skin disease broke out in Taichung and Changhwa in Central Taiwan. The cause of the disease was later identified to be the ingestion of rice-bran oil contaminated with polychlorinated biphenyls (Chen et al., 1980, 1981; Hsu et al., 1984; Masuda et al., 1986). By the end of 1980, the total number of reported cases was about 2000. The local name for the disease was Yu-cheng. The mean consumption of total PCDFs of the Yusho and Yu-cheng patients has been estimated to be 3.3-3.8 mg/person or 400-500 mg of toxic 2,3,7,8-substituted PCDFs per person. (Hayabuchi et al., 1979). Hayabuchi et al. (1979) estimated the daily intake of total PCDFs in the Yusho intoxication to have been 0.9 µg/kg body

weight. Analyses of liver samples taken from the Yusho patients about 18 months after the exposure showed a dramatic decrease in the number of PCDF isomers. Apparently most of the PCDF isomers were metabolized or excreted during the period between exposure and sampling (Rappe et al., 1979). A comparison between the PCDF isomers found in the Yusho oil and the liver samples revealed an interesting relationship. Most of the isomers retained had all lateral positions (2-, 3-, 7-, and 8-) substituted with chlorine (Rappe et al., 1979).

Table 72. Clinical symptomatology of Yusho 1969-1972[a]

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1. Skin (82-87%).
    Acneiform eruptions, districtive hair follicles, red plaques on limbs, dark brown pigmentation of nail, skin, and mucous membranes, itching, sweating of palms.

2. Ocular manifestations (83-88%).
    Increased eye discharge, swelling of the upper eyelids, hyperaemia of conjunctiva, transient visual disturbance.

3. Jaundice (10%).
    No abnormalities of liver function in the majority of cases.

4. Numbness of the limbs, feeling of weakness, muscular spasms (32-39%).
    Reduced sensory and motor nerve conduction velocity in a few cases (9%).

5. Hearing difficulties (18%).

6. Headaches, vomiting, diarrhoea (17-39%).

7. Chronic bronchitis (40%).
    Low serum IgA and IgM, PCB in the sputum.

8. Irregular menstrual cycles (60%).

9. Dark brown skin pigmentation (which gradually fades) of newborn, retarded growth, abnormal teeth number and shape.

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[a] Numbers in brackets refer to per cent of patients exhibiting the symptoms.

       Table 73. Changes in the clinical symptomatology of Yusho
in the years 1968-1978

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1.   Skin lesions.
      All skin symptoms diminished gradually, subcutaneous cyst formation
      still present in some of the most severe cases.

2.   Ocular manifestations.
      Eye discharge, oedema of the eyelids, pigmentation of eyelids and
      conjunctiva, and cyst formation of tarsal gland still present in some
      of the cases.

Table 73.(cont'd) Changes in the clinical symptomatology of Yusho
in the years 1968-1978

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3.   Stomatological alterations.
      Pigmentation of oral mucosa decreased gradually; anomalies in number
      of teeth and shape of the root still present.

4.   Chronic bronchitis correlated in severity with concentration of PCBs
      in sputum and blood.

5.   Serum triglycerides.
      The hyperglycidaemia observed in 1968-1970 returned to a normal level
      by 1973 in females and by 1975 in males.

6.   Mortality.
      Of 737 cases in the Fukuoka region, 51 (6.92%) died between 1968 and
      1978; there were 11 cancer deaths (3 stomach cancer, 2 lung cancer,
      1 breast cancer, 1 liver cancer, 2 malignant lymphoma).

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       The rate of excretion of these toxic PCDF isomers is very slow.
Rappe et al., (1983c) could detect 2,3,4,7,8-pentaCDF in blood plasma
from Yusho patients when the samples were collected 11 years after
exposure. Higher levels were found in blood from Yu-cheng patients one
year after exposure and these analyses also showed a 15-20% reduction
in one year (Rappe, 1984). PCDFs are selectively retained in the
liver, with levels corresponding to the fatty level of the tissue.
They are not found in unexposed controls or in PCB-exposed workers.
PCB levels in Yusho patients were only about two times higher than

those of normal people several years after the outbreak. PCB-exposed
workers had more than 10 times greater PCB blood levels than Yusho
patients, whereas the PCQ levels of these two groups were similar.

Generally a correlation between degree or severity of clinical
signs and the amount of PCDFs retained in the blood exists, whereas
there is no correspondence between the severity of disease and PCB
concentrations in blood.

Mild dermal lesions seen in workers exposed to PCB disappeared
quickly after discontinuation of PCB handling, in contrast to the
persistence of Yusho and Yu-cheng symptoms. Everything thus suggests
that the PCDF contaminant is the causative agent.

Immunological evaluation of patients exposed in 1979 in Taiwan
(Yu-cheng) has been reported by Chang et al. (1981, 1982a, 1982b) and
Wu et al. (1984). Serum immunoglobulin concentrations and lymphocyte
subpopulations were determined in the peripheral blood of 30 patients

exposed to PCBs and 23 healthy individuals. The groups were age and
sex matched. In the patients, serum concentrations of IgA and IgM, but
not of IgG, were significantly decreased. Also the percentages of
total T lymphocytes and T-helper lymphocytes were significantly
reduced, whereas the percentages of T-suppressor cells and B
lymphocytes were not affected (Chang et al., 1981).

In a later report (Chang et al., 1982a), monocyte and
polymorphonuclear lymphocyte (PMN) complement and Fc receptors were
evaluated in peripheral blood from 30 Yu-cheng patients and 23 normal
human subjects. Monocytes and PMNs from patients had significantly
lower percentages of cells bearing immunoglobulin Fc and complement
receptors. The immune system was further investigated by determining
the delayed-type hypersensitivity skin response to streptokinase and
streptodornase, as parameters of cellular immune function. The
response was studied in 30 PCB-poisoned patients and 50 healthy
volunteers. Results of the study showed that 80% of the controls had
positive hypersensitivity skin tests, compared to only 43% of the
patients. The significant suppression of cellular immunity correlated
with the severity of the dermal lesions; the size of the
hypersensitivity skin reaction was negatively correlated with the
dermal lesions and also with PCB concentrations in whole blood (Chang
et al., 1982b). More recently, the delayed-type hypersensitivity
response to tuberculin was reported in 83 PCB-poisoned Yu-cheng
patients and in 30 age-and sex-matched healthy controls. Compared to
a positive response rate of 74% in the control group, the patients had
a significantly lower skin response of 48% (40 out of 83 patients). In

contrast to the delayed-type hypersensitivity response, the in vitro proliferation responses of peripheral blood lymphocytes treated with phytohaemagglutinin and pokeweed mitogens, as well as tuberculin, were significantly enhanced (Wu et al., 1984).

There are also indications of immunosuppression in the Yusho poisoning. Serum IgA and IgM levels decreased considerably within 2 years after the onset of the disease. Respiratory involvement included bronchiolitis, and respiratory distress was often exacerbated by viral or bacterial infection (Shigematsu et al., 1978).

The symptomatology of Yusho has been summarized by Reggiani (1983b) and is to be found in Tables 72 and 73. It is similar to that of Yu-cheng, but there are differences such as the frequency of transient visual disturbances, hearing difficulties, and a persistent bronchitis.

12.   EVALUATION OF HEALTH RISKS FROM THE EXPOSURE TO CHLORINATED DIBENZO-P-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs)

12.1   Introduction

In order to evaluate the human health risk of PCDDs and PCDFs, it is necessary to know both the levels of human exposure and the corresponding human health effects.

Human exposure assessment is complex. Several approaches may be taken to estimate it, such as the following.

(a)   Use of standard physiological models of inhalation, ingestion, and dermal absorption. Data requirements include detailed information on ambient levels in environmental media and food, and on bioavailability.

(b)   Intake estimates based on simple pharmacokinetic models and known levels in human tissue for accurate assessment of exposure. Detailed knowledge is also required of the uptake, distribution, metabolism, and elimination of PCDDs and PCDFs in humans.

12.2   Exposure Assessment

12.2.1   Sources of contamination

The main sources of PCDDs and PCDFs that have so far been identified are contaminated commercial chemicals (see section 3.3), emissions from combustion sources (see section 3.5), and disposal of

industrial wastes containing PCDDs and PCDFs (sections 3.4, 3.5.9, and 3.5.10).

In some cases estimates of the relative contribution of these sources can be generated on a local basis. However, the data and methods available today do not allow firm conclusions with regard to the relative quantitative importance of these sources on a nation-wide or world-wide basis.

12.2.2 Ambient levels

The limited data available indicate very low (fg/m^3) background levels in ambient air of the 2,3,7,8-tetra-, penta-, and hexachlorinated PCDDs and PCDFs. (If hepta- and octachlorinated congeners are included, pg/m^3 levels are noted).

The few data available indicate that the 2,3,7,8-tetra-, penta-, and hexachlorinated PCDDs and PCDFs are unlikely to occur in finished drinking-water, even at a level of 1 pg/litre (see section 5.2). In all soil and sediment samples analyzed (both from industrialized and non-industrialized areas), PCDDs and PCDFs were identified at levels ranging from a few ng/kg to several hundred ng/kg (the latter in sediments and in urban soil).

There are no available data on background levels of PCDDs and PCDFs in vegetation in the general environment.

Levels of up to 50 ng/kg of the 2,3,7,8-tetra-, penta-, and hexachlorinated PCDDs and PCDFs (principally the tetra- and pentachlorinated congeners) have been found in fish from the general environment. For the most part these have been detected in fatty or bottom-feeding fish (see section 4.4.2).

Data from terrestrial organisms are inadequate for estimation of background levels (see section 4.4.3). Three samples of pooled cow's milk showed a maximum of 100 pg/kg of the 2,3,7,8-tetra-, penta-, and hexachlorinated PCDDs and PCDFs in whole milk (see section 5.4). Data on contamination of other commercial foods are also very limited. Analyses of several samples of chicken and pork have shown contamination with highly chlorinated congeners at about 5-30 ng/kg. The congener profile differs from that noted in aquatic organisms (see section 5.4).

The data available are not sufficient for assessing the total exposure of general populations. They are sufficient to perform a limited evaluation of exposure for local populations. Based on the

environmental levels discussed above and the usual assumptions regarding intakes of foodstuffs, air, and water, food is more likely to be a significant source of PCDD/PCDF exposure than air, while drinking-water is likely to be of much less concern.

12.2.3   Routes of exposure

Human adipose tissue contains 2,3,7,8-tetra-, penta-, and hexachlorinated PCDDs and PCDFs. This contamination is presumably due to exposure at the ambient concentrations noted in the preceding sections.

In addition, infants may be exposed through breast milk, and small children may also be exposed through ingestion of contaminated soil. However, this latter route of exposure, in most instances, is likely to be of concern only in heavily contaminated areas.

Some populations have been at special risk through exposure in industrial accidents (and their clean-up) that have occurred during the normal production and use of chlorphenols and phenoxy herbicides and PCBs. In these situations inhalation and dermal contact are the exposure routes of greatest concern. However, quantitative information on the nature and concentration of contaminants is not available.

Based on the environmental levels discussed above and the usual assumptions regarding physiological and intake parameters, ingestion is likely to be the exposure route of greatest concern. Inhalation of

ambient air is not likely to be a problem, although inhalation of heavily contaminated air may make a significant contribution to exposure. In general, it is not possible, at present, to estimate the relative contribution of dermal exposure.

12.2.4   Bioavailability

No bioavailability data from studies in humans are available. From animal studies, it is clear that bioavailability of PCDDs and PCDFs following ingestion depends on the matrix ingested. Table 46 summarizes the data available on oral intake.

Studies on hairless rats indicate that dermal exposure through intact skin from contact with contaminated soil is about 1 to 2%. No data are available for inhalation exposure.

12.3   Animal Data

12.3.1 Toxicokinetics of 2,3,7,8-TCDD

     Studies on rodents given single or repeated oral doses of
2,3,7,8-TCDD have shown that 50% or more of the administered amount is
absorbed from the gastrointestinal tract in rats, guinea-pigs, and
hamsters, but less than 30% in mice (Table 40). The reported
half-lives for elimination were in the ranges 12-31 days for rats,
mice, and hamsters and 22-94 days for guinea-pigs (Table 41). However,
most of these studies have been performed at toxic doses. The
half-life of 2,3,7,8-TCDD in primates has not been well established,
but available data for the rhesus monkey suggest an apparent half-life
in the adipose tissue of about 1 year.

     2,3,7,8-TCDD does accumulate in animal tissues. In rodents
accumulation occurs predominantly in the liver and adipose tissue
(Tables 42 to 44). In rhesus monkeys (Table 45), high levels of
2,3,7,8-TCDD are recovered from the adipose tissues, liver, skin, and
muscles.

     At a daily dose of 1 ng 2,3,7,8-TCDD/kg body weight for 2 years,
rats accumulated 540 ng 2,3,7,8-TCDD/kg body weight in the liver where
some morphological changes were also observed. Similar levels were
found in beach mice that had been exposed to 2,3,7,8-TCDD soil levels
ranging from 10-710 ng/kg. Total body exposure of animals to
2,3,7,8-TCDD in soil at such concentrations may thus result in tissue
levels that have been demonstrated to cause effects in experimental
animals.

     TCDD is largely eliminated in the faeces, although some urinary
excretion occurs. The hamster has a higher urinary elimination than
other species studied.

     Transformation of 2,3,7,8-TCDD to more polar metabolites occurs
in all animal species investigated (see section 6.2 and Table 62).
Elimination of metabolites from tissues into faeces and urine occurs
rapidly in all of these species except in the case of the guinea-pig.
Known metabolites are much less toxic than the parent compound.

12.3.2 Toxicokinetics of PCDDs and PCDFs, other than TCDD

     Animal data on the toxicokinetics of pure PCDDs other than
2,3,7,8-TCDD are limited. PCDFs have been more extensively studied in
this respect. The half-life for 2,3,7,8-TCDF has been reported to be
in the range of 2-8 days for rats, mice, and rhesus monkeys and more
than 20 days for guinea-pigs (Table 65). Studies on rats have shown
that 2,3,4,7,8-pentaCDF is more highly retained than is 2,3,7,8-TCDF

(65% and 3.8%, respectively, after 5 days).

Tissue retention data of PCDDs and PCDFs in various species exposed to synthetic mixtures or to environmental samples containing PCDDs and PCDFs show a high variability in retention time between congeners with or without chlorine substitution in all the positions 2,3,7, and 8.

12.3.3 Toxic effects of 2,3,7,8-TCDD

The toxic and biological effects resulting from exposure to 2,3,7,8-TCDD are dependent on a number of factors, including the species, strain, age, and sex of the animals used. The toxic responses observed in several animal species include body weight loss, hepatotoxicity, porphyria, dermal toxicity, gastric lesions, thymus atrophy and immunotoxicity, teratogenicity, reproductive effects, and carcinogenicity. TCDD induces a wide spectrum of biological effects including enzyme induction and vitamin A depletion. The complete spectrum of toxic and biological effects is not usually observed in any single animal species. The two most characteristic toxic effects observed in all laboratory animals are body weight loss and thymus atrophy and immunotoxicity. Chloracne and related dermal lesions are the most frequently noted signs of 2,3,7,8-TCDD toxicosis in humans; dermal lesions are also observed in rhesus monkeys, hairless mice, and rabbits. In contrast, rats, most strains of mice, guinea-pigs, and hamsters do not develop chloracne and related dermal toxic lesions after exposure to 2,3,7,8-TCDD. Many of the observed toxic lesions are either hyperplastic/metaplastic or hypoplastic, and primarily affect epithelial tissues.

Reproductive toxicity has been reported in rhesus monkeys: the lowest-observed-effect level (LOEL) was calculated to be 1 to 2 ng/kg body weight per day. A no-observed-effect level (NOEL), or possibly a LOEL, of 1 ng/kg body weight per day for reproductive effects in rats has been discussed (Murray et al., 1979; Nisbet & Paxton, 1982).

If the cancer studies in rats conducted by Kociba et al. (1978) and by the NIH (1982a,b) are compared, it is evident that the liver tumours, including hepatocellular carcinomas, are produced at similar dose levels. Although an increased incidence of tumours in other organs was observed by the NTP, and by Kociba et al. (1978), the other target organs varied in the two studies. This may be caused, in part, by differences in dosing (gavage versus exposure in ground feed) and by differences in strains. In the Kociba study, doses of 10 ng/kg body weight caused an increased incidence of neoplastic (hyperplastic) nodules in females, and doses of 1 ng/kg body weight resulted in foci

or areas of hepatocellular alteration (swollen hepatocytes). At these
dose rates in experimental groups, the incidence of certain
hormone-dependent tumours was lower than in the control animals,
suggesting endocrine changes induced by 2,3,7,8-TCDD. Based on these
animal studies and on available human data IARC (1982 suppl. 4)
concluded that TCDD showed sufficient evidence for carcinogenicity in
animals, but inadequate evidence for carcinogenicity in humans.

TCDD does not appear to have mutagenic properties, and is,
therefore, not likely to be genotoxic. Thus, it is assumed to be
carcinogenic through an indirect (epigenetic) mechanism.

12.3.4 Toxic effects of PCDDs and PCDFs, other than TCDD

Several other PCDDs and PCDFs cause signs and symptoms similar to
those of 2,3,7,8-TCDD, but there is a wide variation with regard to
potency (Tables 56, 62). In summary, there are 12 isomers that display
high toxicity, i.e., the tetra-, penta-, hexa-, and heptaCDDs and CDFs
with four chlorine atoms in the symmetrical lateral positions 2,3,7,
and 8. A mixture of two hexaCDDs (1,2,3,7,8,9- and
1,2,3,6,7,8-hexaCDD) has been demonstrated to possess carcinogenic
properties in long-term animal studies, but at higher doses than those
used in the study of TCDD. Unsubstituted dioxin and 2,7-diCDD failed
to demonstrate carcinogenic properties.

The relative toxic and biological potencies of PCDDs and PCDFs
have been estimated using short-term studies in rats and mammalian
cell cultures. Endpoints used include inhibition of body weight gain,
thymic atrophy, enzyme induction, teratogenicity, acnegenic response,
and keratinization. In the absence of long-term toxicity data, results
obtained from such short-term tests are at present the only source for
ranking the toxicity for human risk assessment.

When investigated, mixtures of these compounds have shown
additive or less than additive responses.

12.3.5 Review of species differences

There are marked species differences in the susceptibility to the
biological and toxic effects elicited by 2,3,7,8-TCDD. For example,
the oral LD_{50} values range from 0.6 µg/kg body weight in

guinea-pigs, to 5051 µg/kg body weight in Golden Syrian hamsters
(Table 47); moreover, pronounced differences in LD_{50} values have

also been reported in different strains of the same species (e.g.,

rats and mice). The toxicity and toxicokinetics of TCDD in monkeys
most closely resemble the effects observed in human beings. However,
the tremendous variation in species and strain sensitivity to
2,3,7,8-TCDD and related compounds cannot be explained by the observed
toxicokinetic differences. There is evidence in inbred mice, that the
cellular levels of the Ah receptor correlate, in part, with
susceptibility to the biological and toxic effects of these compounds.
The receptor has also been identified in other species, including
human beings. However, interspecies comparison of cellular Ah receptor
levels do not explain their differences in sensitivity to
2,3,7,8-TCDD; this is consistent with complex as yet unknown
mechanisms of toxicity that involve multiple factors in addition to
the Ah receptor.

12.4 Human Health Effects

12.4.1 PCDDs

     Exposure of the general population is to small amounts of PCDDs
and PCDFs in complex mixtures and these have not been associated with
disease. In a few incidents workers and others have been exposed to
larger amounts of a limited number of these compounds, e.g., Seveso
and in Yusho disease.

     For occupational and accidental exposure the most prominent
clinical effect has been chloracne. Other effects (Table 64) have been
noted, but, apart from chloracne and perhaps minor functional
disorders, none has been persistent.

     In some, but not all mortality studies, an increased incidence of
cancer at different sites has been claimed, but the small numbers of
cases limit confidence in the findings.

     The overall impression from the follow-up studies is that even
severe acute systemic effects of TCDD are usually reversible, except
for chloracne, or markedly improved over time following cessation of
exposure. In Seveso, the only clear-cut adverse health effect recorded
has been chloracne. 193 cases of chloracne occurred in 1976 and 1977,
and 20 of those still presented active symptoms in 1984. Many studies
have been performed to find possible links between exposure and health
effects in civilians or military personnel exposed to Agent Orange in
Viet Nam. However, the information available to date does not allow
definite conclusions to be drawn with regard to effects on human
reproduction or any other significant health effects (see section
9.2).

In a number of studies, exposed populations and various control groups have been compared by measuring serum lipids, liver function tests, and other variables. Although certain statistically significant differences have been reported there, and also in isolated case reports, lack of uniformity, various technical shortcomings, and the inability to exclude confounding factors means that the results have been inconclusive.

In the Missouri (USA) incident, children who showed acute illness when the contamination occurred in 1971 are now reportedly in good health. Epidemiological studies in Missouri on populations exposed to lower concentration over longer periods of time have so far not revealed any significant health effects. Although no clinical illness was observed, there were indications of an effect on the cell-mediated immune system.

The ranges of health effects produced by TCDD in human beings have yet to be defined. It can be concluded that the data from human exposure and effects, when taken together, do not allow any determinations of dose/effect or dose/response relationships in human beings.

In spite of many clinical and follow-up studies, no clearcut persistent systemic effects have been delineated, except for chloracne. In the light of present information, it seems unlikely that permanent, severe, and debilitating toxicological sequelae are inevitable after exposure to TCDD.

12.4.2 PCDFs

The only well documented intoxications with PCDFs in human beings are the two instances of contamination of rice oil with PCDFs, PCBs, and PCQs, i.e., Yusho in Japan (1968) and Yu-cheng in Taiwan (1979) (see section 11.1). In total, several thousand people were acutely intoxicated. The summarized data makes it most likely that the causative agent was PCDFs. The general symptomatology was similar to that found in intoxications with TCDD. The differences may reflect intensity in exposure and the ages and sex of the exposed human beings. Attempts to estimate the average daily intake of PCDFs over several months in Yusho patients indicated a figure of 0.9 µg/kg body weight of total PCDFs, 0.1-0.2 µg/kg of 2,3,7,8-substituted tetra-, penta-, and hexaPCDFs, together with 157 µg PCBs and 148 µg PCQs/kg body weight (Hayabuchi et al., 1979). The lowest dose causing disease was estimated to be 0.6 mg total PCDFs per person over 30 days, corresponding to a daily dose of 0.05-0.1 µg/kg body weight of 2,3,7,8-substituted PCDFs. However, the data available are not

sufficient to permit any conclusions as to what dose might be safe for human intake.

12.4.3 Human body burden and kinetics

     In human fat, background levels of TCDD up to 20 ng/kg have been found in the general population with no known specific exposure, but higher levels have been reported in some cases without evidence of disease. None of these populations have been randomly sampled. The more highly chlorinated other PCDDs and PCDFs, especially octaDD, also occur in these samples (see Tables 29, 30). Averages values seem to increase with age.

     In special situations, higher levels (in the low µg/kg range) have been found that have not been associated with disease.

     In the Yusho and Yu-cheng incidents, symptoms were noticed at higher levels of PCDFs, e.g., 2,3,4,7,8-pentaCDF was found at 6.9 µg/kg fat tissue one year after the exposure to contaminated rice oil.

     Based on the very limited data available, the levels of, for instance, 2,3,4,7,8-pentaCDF in the general population, seem to be two orders of magnitude lower than the levels associated with the Yusho disease.

     No such comparisons can be made for PCDDs.

     Limited data indicate that those isomers chlorinated at the 2,3,7, and 8 positions are selectively retained, except for TCDF.

     A half-life for TCDD in human beings of 5 years has been indicated by one experimental study. In another study half-lives in the range of 2-6 years were estimated for 1,2,3,6,7, 8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, octaCDD, 1,2,3,4,6,7,8-heptaCDF, and octaCDF. These data need to be expanded since they are based on studies in only two subjects and since the toxicokinetics of these types of compounds may not simply be controlled by first-order kinetics. However, even if there are limitations in the present data, it is apparent that the half-lives of these compounds are in the range of one or more years.

     These reported half-lives for human beings are very different from those reported in rodents. However, animal experiments have usually been performed with toxic doses. Furthermore, animals with a short life span have a higher metabolic rate, thus shorter half-lives could be expected.

The PCDDs and PCDFs are predominantly stored in fat, but they are also excreted in milk (Table 39) and pass the placenta. They also appear in the blood and vital organs at lower concentrations. The distribution between different tissues in human beings is not at present clear, although it has been suggested that the ratio between fatty tissue and liver is higher in human beings than that in rodents.

However, this conclusion is based on very limited data from autopsy specimens. Whether this is relevant for the general population remains to be seen.

The intake route for human beings is at present not very well delineated, but it has been assumed that intake from food is the main route. However, the human infant represents a special case; because of transplacental transfer of these compounds, the neonate might be expected to be exposed in utero. Levels measured so far in human milk suggest that this food might be an important source of these compounds.

No data are available regarding what dose of PCDDs is toxic to human beings. However, in the Yusho and Yu-cheng episodes, total intakes of total PCDFs in the range 3.3-3.8 mg/person and total intakes of 2,3,7,8-substituted PCDFs in the range 400-500 µg/person were associated with the disease. No good data are available as to what intakes occurred without causing disease.

12.5 General Conclusions

PCDDs and PCDFs occur throughout the environment and we all probably carry a body burden of them. They have sometimes produced complex toxic effects following occupational and accidental exposure.

Based on the Yusho disease and experiments in sensitive species of monkeys, and making assumptions about the relative potencies of PCDDs and PCDFs, human beings and certain monkey species may have comparable sensitivity to these compounds. However, the uncertainties related to the real dose received by human beings and the difficulties of assessing toxic effects other than chloracne in our species prevent a firm conclusion as to the relative resistance of human beings to the toxic effects of these compounds. Exposure should be reduced to levels as low as are reasonably practicable.

13. RECOMMENDATIONS

1.   Analytical interlaboratory validation and round-robin studies
      using standardized quality assurance and quality control

procedures are needed to improve analytical methodology.

Sampling strategy and analytical procedures and data
interpretation should be optimized and standardized before
undertaking surveys.

2.   Further information is required about the origins and
       environmental distribution and fate of PCDDs and PCDFs. Further
       monitoring data, including time trends and determinations of
       isomer patterns, are required for environmental levels of PCDDs
       and PCDFs, especially for food, ambient air, and sediments.

3.   Data should be obtained about the effects of PCDDs and PCDFs on
       environmental biota.

4.   More information is required about the bioavailability of PCDDs
       and PCDFs from different matrices in the environment and from the
       diet. Exposure from these sources should be correlated with
       agricultural and industrial practices.

5.   Simpler and less expensive methods suitable for screening should
       be developed and validated.

6.   Studies to determine the mechanisms of toxicity of PCDDs and
       PCDFs are needed to support an evaluation of the differences in
       effects between species and to allow extrapolation to human
       beings.

7.   Further investigation of immunotoxicity is important, including
       cytotoxic T lymphocyte function. Studies of the effects of
       perinatal exposure and of the duration of actions on the immune
       system are important.

8.   Long-term toxicity studies, including multigeneration
       reproductive studies, in different species with three of the most
       widespread PCDDs and PCDFs, namely 2,3,4,7,8-pentaCDF,
       1,2,3,7,8-pentaCDD, and octaCDD, should be carried out.

9.   Because humans are exposed to complex mixtures of PCDDs and
       PCDFs, test systems, including techniques applicable to
       evaluating human tissues, should be further developed and
       validated for the toxic potency of these compounds and other
       mixtures. These systems can be used to study mechanisms of
       action, structure activity relationships, and interactive
       effects.

10. Investigations to examine the body burden and to correlate it with clinical effects and laboratory findings are indicated. Follow-up studies of previously exposed groups are important.

14. EVALUATIONS BY INTERNATIONAL BODIES AND THE CONCEPT OF TCDD EQUIVALENTS

14.1 International Evaluations

IARC evaluated the carcinogenic risk of TCDD to man (IARC, 1977, 1982) and concluded that there was sufficient evidence that it was carcinogenic to animals, but that the data for carcinogenicity to human beings was inadequate. None of the other PCDDs or PCDFs have been evaluated by IARC.

Regulatory standards for TCDD and mixtures containing TCDD, established by national bodies in different countries and the European Economic Community, are summarized in the Legal File of the International Register of Potentially Toxic Chemicals (IRPTC, 1987).

14.2 Methodologies Used in Assessment of Risk from PCDDs and PCDFs

14.2.1 Individual Congeners

As shown in this monograph, sufficient high quality data for assessing human health risks exist only for TCDD. For the other congeners and isomers where data do exist, they are generally derived from studies using acute exposures in experimental animals and/or from in vitro tests.

The several risk evaluations on TCDD from various countries have utilized the long-term ocogenic rat studies (Kociba et al., 1978) or the reproduction studies on rats (Murray et al., 1979) or monkeys (Schantz et al., 1979). Mathematical models have been applied to the cancer data and "virtually safe doses" between 0.006 and 0.028 pg/kg body weight per day have been calculated (Kimbrough, 1984). The biological relevance of such models has been questioned, since TCDD has not been shown to be genotoxic, and has been found to be a strong promoter of liver tumours in a two-stage precarcinogenesis study (Pitot et al., 1980) To avoid the use of mathematical models, several evaluations have used safety factors in the range of 100 to 1000 applied to the assumed no-effect, or lowest-observed-effect levels in the cancer study of Kociba et al. (1978) or the reproduction studies of Murray et al. (1979) or Schantz et al. (1978). Using this methodology "tolerable daily intakes" have been calculated for human beings in the range of 1-10 pg/kg body weight (Denmark, 1984; Ontario,

1985; US EPA, 1985; Ahlborg & Victorin, 1987).

14.2.2 Mixtures of PCDD and PCDF congeners and isomers -
concept of TCDD toxic equivalents.

     The results of recent isomer-specific analyses of such diverse
environmental samples as emissions from the combustion of hazardous
industrial and municipal wastes, soil, industrial process wastes,
human adipose tissue, and milk indicate that the majority of the 75
CDD and 135 CDF isomers can be detected. Humans are therefore exposed

to complex mixtures of these environmental contaminants (sometimes
2,3,7,8-tetraCDD is only a minor component), and the level of risk
from such exposures must be assessed.

     In the absence of long-term whole animal tests on complex
mixtures of PCDDs and PCDFs, as well as similar studies on individual
isomers and/or congeners, several models have been proposed to relate
the toxicity of environmental mixtures to the well studied isomer
2,3,7,8-TCDD. The results from these models are presented as "TCDD
Toxic Equivalents". A summary of some models which have been published
is given in Table 74. The toxic potencies used, relative to TCDD, are
shown. The scientific basis for deriving these relative toxicities is
somewhat different for each model. The Swiss (Switzerland, 1982) and
Danish (Denmark, 1984) models are based essentially on the relative
potency for AHH induction, whereas the German (Germany, 1985) and
Canadian models (Ontario, 1985) are based on a weighting of all
available quantitative data. The USA model (US EPA, 1987) utilizes
primarily the relative carcinogenic potencies of TCDD and hexaCDD,
with consideration given to other relevant quantitative data. A
discussion of the structure-activity relationships and relative
biological activities of many PCDD and PCDF isomers is given in
sections 7 and 10.

     Data requirements, and assumptions made in the application of
these models to various environmental mixtures have been reviewed by
US EPA (1987) and Ontario (1985). Suter-Hofmann & Schlatter (1985) fed
toluene extracts of acid-washed particulates from a municipal waste
incinerator to Sprague Dawley rats at levels in the diet corresponding
to 4, 12, and 24% of particulates. The calculated daily TCDD intakes
at these doses were 4.8, 14.4, and 28.8 ng/kg body weight. Depending
upon which model was used from Table 74, the estimated intake of PCDDs
and PCDFs would be 48-300 (4% particulates), 144-900 (12%
particulates), and 288-1800 ng TCDD-equivalents/kg body weight per day
(24% particulates). No mortality was noted in the groups fed 24%
particulates, but at this dose body weight gain was depressed in

females and depressed thymus and increased liver weights were noted in both sexes. No adverse affects were seen at the 4% and 12% dose levels. From the known toxicity of TCDD, more severe effects would have been expected from the calculated dose of between 288 and 1800 ng TCDD-equivalents/kg body weight per day. These data support the conclusion that the application of TCDD-equivalence models may over-estimate the inherent risk from exposures to dioxin-containing environmental mixtures, depending largely upon the assumptions used in deriving the model. However, a definitive conclusion on this point awaits further research (US EPA, 1987) (see section 13).

Table 74. Examples of TCDD-equivalence models

Relative PCDD and PCDF toxicities in model

Compound	Denmark (1984)	Ontario (1985)	Olie et al. (1983)	United States (US EPA 1987)	Switzerland (1982)	Germany (1985)
MonoCDD	0				0.001	0
DiCDD	0				0.001	0
TriCDD	0				0.01	0
TetraCDD-2,3,7,8	1.0	1.0	1.0	1.0	1.0	1.0
-non 2,3,7,8	0.01	0.01	1.0	0.01	0.01	
PentaCDD-1,2,3,7,8			0.1			0.01
-all 2,3,7,8	0.1	0.1	0.1	0.5	0.1	
-non 2,3,7,8	0.01	0.1	0.1	0.005	0.1	
HexaCDD-1,2,3,4,7,8						0.1
-1,2,3,6,7,8						0.01
-1,2,3,7,8,9						0.01
-all 2,3,7,8	0.1	0.1	0.1	0.04	0.1	
-non 2,3,7,8			0.1		0.1	

Compound	Denmark (1984)	Ontario (1985)	Olie et al. (1983)	United States (US EPA 1987)	Switzerland (1982)	Germany (1985)
(continued)	0.01	0.1		0.0004		
HeptaCDD-1,2,3,4,6,7,8						0.01
-all 2,3,7,8	0.01	0.01	0.1	0.001	0.1	
-non 2,3,7,8	0.001	0.01	0.1	0.00001	0.1	
OctaCDD	0.001	0.0001	0	0		
MonoCDF						0.0001
DiCDF						0.0001
TriCDF						0.01

Table 74 (contd).

Relative PCDD and PCDF toxicities in model

Compound	Denmark (1984)	Ontario (1985)	Olie et al. United States (1983)	(US EPA 1987)	Switzerland (1982)	Germany (1985)
TetraCDF-2,3,7,8	0.1	0.5	0.1	0.1	0.1	0.1
-non 2,3,7,8	0.01	0.5	0.1	0.001	0.1	
PentaCDF-1,2,3,7,8						0.2
-2,3,4,7,8						0.2
-all 2,3,7,8	0.1	0.5	0.1	0.1	0.1	
-non 2,3,7,8	0.01	0.5	0.1	0.001	0.1	
HexaCDF-1,2,3,4,7,8						0.2
-1,2,3,6,7,8						0.05
-1,2,3,7,8,9						
-2,3,4,6,7,8						0.1
-all 2,3,7,8	0.1	0.1	0.1	0.01	0.1	
-non 2,3,7,8	0.01	0.1	0.1	0.0001	0.1	
HeptaCDF-1,2,3,4,6,7,8						0.01
-1,2,3,4,7,8,9						
-all 2,3,7,8	0.01	0.01	0.1	0.00001	0.1	

```
          -non 2,3,7,8        0.1
0.001                0.01            0.00001
    OctaCDF                     0
0.001                0.0001         0
```

It must be recognized that an approach such as the use of
"TCDD-equivalents" must be regarded as an interim procedure for the
measurement of the toxicity of environmental samples in the absence of
long-term toxicity data on specific PCDD and PCDF isomers and mixtures
of these compounds. At present it is an imprecise evaluation
methodology with many data gaps in the supporting data base.

The TCDD-equivalent models have two major sources of uncertainty,
i.e., firstly, the unanswered scientific questions related to the
toxicity of TCDD itself, and secondly, the lack of data on the other
PCDD and PCDF congeners and isomers that would permit a more accurate
determination of the potency of these chemicals relative to TCDD. The
research recommendations in this document address some of these
concerns. As such data become available, TCDD-equivalent models must
be continuously updated and risk assessments based on the present
models (Table 74) considered only as interim evaluations.

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RESUME ET RECOMMANDATIONS

1. Résumé

1.1   Sources

     Les dibenzo-para-dioxines polychlorées (PCDD) et les dibenzofurannes polychlorés (PCDF) constituent deux séries d'hydrocarbures aromatiques tricycliques halogénés de propriétés physico-chimiques très voisines, présentes un peu partout dans l'environnement. Leur origine n'est pas naturelle et ils ne sont pas produits intentionnellement. On connait 75 isomères (isomérie de position) pour les PCDD et 135 pour les PCDF.

     Les sources les plus importantes de contamination par ces deux séries de composés sont les suivantes:

-       contamination de produits chimiques du commerce, par exemple chlorophénols et dérivés, PCB;
-       incinération de déchets municipaux, de déchets dangereux et de déchets d'hôpitaux, ainsi que de boues provenant du traitement des effluents;
-       fonctionnement des véhicules automobiles;
-       utilisation de combustibles fossiles;
-       surchauffe et émissions de foyers faisant intervenir des béniphyles polychlorés (PCB);
-       élimination de déchets industriels provenant, par exemple, de la production de chlorophénols et de leurs dérivés, du traitement du bois par le chlorophénol, de l'emploi de PCB liquides dans l'appareillage électrique et du traitement du papier et de la pâte à papier.

1.2 Concentrations dans l'environnement et voies d'exposition

     D'après les quelques données dont on dispose, la concentration de

ces composés serait très faible dans l'air, le sol et les sédiments, de l'ordre du femtogramme (fg) par mètre cube dans l'air et du nanogramme (ng) par kilogramme dans le sol et les sédiments. On a observé des concentrations allant jusqu'à 50 mg/kg, aussi bien pour les PCDD que pour les PCDF, dans certains organismes aquatiques à l'état naturel. On dispose de très peu de données sur la contamination de l'eau de boisson et des denrées alimentaires vendues dans le commerce.

Pour ce qui est de la population en général, l'exposition à ces composés semble principalement associée à la chaîne alimentaire.

Certains travailleurs peuvent être très exposés lorsqu'ils sont affectés à la production, la mise en oeuvre ou à la destruction de matériaux contenant des PCDD ou des PCDF, ainsi que leurs précurseurs. Pour ce personnel, l'inhalation et les contacts cutanés constituent les principales voies d'exposition à prendre en compte.

1.3 Toxicocinétique, biotransformation et surveillance biologique

La biodisponibilité des PCDD et des PCDF dépend de la matrice au sein de laquelle ils sont incorporés et de la voie d'exposition. On ne dispose pour aucune des espèces en cause de données sur la biodisponibilité par inhalation.

On ignore la quantité absorbée par l'homme en cas d'exposition, par quelque voie que ce soit.

Les études sur des rongeurs à qui l'on a administré une ou plusieurs doses de 2,3,7,8-TCDD par voie orale montrent qu'environ la moitié de la dose administrée est absorbée dans les voies digestives. La demi-vie d'élimination se situe, d'après la littérature, entre 12 et 94 jours chez les rongeurs. Chez le singe rhésus, la demi-vie de la 2,3,7,8-TCDD est d'environ 1 an dans le tissu adipeux.

Les observations concernant la toxicocinétique des PCDD chez les animaux sont limitées, en dehors du cas de la 2,3,7,8-TCDD. Pour ce dérivé, on a observé une demi-vie de l'ordre de 2 à 8 jours chez le rat, la souris et le singe et de plus de 20 jours chez le cobaye. Chez le rat, on a constaté que la pentachlore-2,3,4,7,8 dibenzofuranne s'élimine moins rapidement que la 2,3,7,8-TCDD.

Les études sur la rétention tissulaire des PCDD et des PCDF chez diverses espèces exposées à des mélanges synthétiques ou à des échantillons prélevés dans l'environnement et contenant des PCDD ou

des PCDF montrent que la durée de rétention des divers analogues est
très variable selon qu'ils sont ou non substitués en 2, 3, 7, ou 8 par
un atome de chlore.

D'après quelques observations effectuées sur l'homme, la demi-vie
serait de 2 à 6 ans pour certains PCDD et PCDF substitués en 2, 3, 7
et 8.

Les PCDD et les PCDF s'accumulent principalement dans les
graisses; ils sont également excrétés dans le lait et traversent la
barrière placentaire. On en trouve également à faible concentration,
dans le sang et les organes vitaux.

La distribution tissulaire chez l'homme est encore mal élucidée,
encore qu'il semble que le rapport des concentrations dans les tissus
adipeux et dans le foie soit plus élevé que chez les rongeurs.

Dans l'ensemble de la population, on observe une concentration
résiduelle de PCDD dans le tissu adipeux qui atteint 20 mg/kg, en
l'absence de toute exposition particulière connue; mais des chiffres
plus élevés ont été signalés quelquefois, sans aucun signe de maladie.
Dans les deux cas, les groupes de population étudiés n'avaient pas été

constitués par sondage aléatoire. Dans ces échantillons, on a observé
les PCDD et les PCDF les plus substitués par du chlore, en particulier
des octachlorodibenzo-p-dioxines. La concentration tissulaire des TCDD
avait tendance à augmenter avec l'âge.

1.4 Effets sur la santé

1.4.1 Animaux

Les effets toxiques et biologiques découlant de l'exposition aux
2,3,7,8-TCDD dépendent de divers facteurs, dont l'espèce, la souche,
l'âge et le sexe des animaux d'expérience. Entre autres signes de
toxicité observés chez plusieurs espèces animales, on note une perte
de poids, une hépatoxicité, une porphyrie, une atteinte cutanée, des
lésions gastriques, une atrophie du thymus, une atteinte du système
immunitaire, des effets tératogènes, des effets sur la reproduction et
des effets cancérogènes. Les TCDD exercent des effets biologiques
extrêmement divers, notamment l'induction de certaines enzymes et une
carence en vitamine A. Ces effets ne sont pas tous observés chez la
même espèce animale. Les effets toxiques les plus caractéristiques
qu'on retrouve chez tous les animaux de laboratoire sont la perte de
poids, l'atrophie du thymus et l'immunotoxicité. Chez l'homme, une
chloracné et des lésions cutanées de ce type constituent les signes

les plus fréquents d'une intoxication par les 2,3,7,8-TCDD; on observe également des lésions cutanées chez le rhésus, la souris glabre et le lapin. En revanche, l'exposition au 2,3,7,8-TCDD ne provoque ni chloracné ni lésions cutanées de ce type chez la plupart des rongeurs. De nombreuses lésions toxiques s'observent principalement au niveau des tissus épithéliaux.

Des effets ont été observés sur la reproduction chez le rhésus et chez le rat. La plus faible dose exerçant un effet serait de l'ordre de 1-2 ng/kg de poids corporel par jour. Dans deux études de cancérogénicité chez le rat, on a obtenu un épithélioma hépatocellulaire avec des doses respectives d'environ 0,1 et 0,01 µg/kg de poids corporel par jour. Une dose réduite à 0,001 µg/kg de poids corporel se traduit par une modification des hépatocytes au niveau de certains foyers ou territoires. L'incidence de certaines tumeurs à déterminisme hormonal était plus faible que chez les animaux témoins.

Apparemment, les TCDD n'ont pas de propriétés mutagènes et ne devraient donc pas être génotoxiques. Il faut donc admettre que les effets cancérogènes s'expliquent par un mécanisme indirect.

Plusieurs autres PCDD et PCDF déterminent des manifestations analogues à celles que provoquent les 2,3,7,8-TCDD, mais leur activité est très inégale. On connaît 12 isomères qui présentent une toxicité plus élevée, à savoir les tétra-, penta-, hexa-et heptaCDD ou CDF qui possèdent quatre atomes de chlore symétriques deux à deux, en poisition 2,3,7 et 8. Des études prolongées sur l'animal ont révélé

l'action cancérogène d'un mélange de deux hexachlorodibenzo-p-dioxines (1,2,3,7,8,9-et 1,2,3,6,7,8-hexaCDD), mais en présence de doses plus élevées que celles qu'on avait utilisées dans l'étude des TCDD. La dibenzo-p-dioxine et la dichloro-2,7 dibenzo-p-dioxine ne se sont pas révélées cancérogènes. Les activités relatives, toxiques et biologiques, des PCDD et des PCDF ont été estimées au moyen d'études de courte durée chez le rat et en culture de cellules mammaliennes.

Selon les espèces, les animaux présentent une sensibilité très inégale aux effets biologiques et toxiques des PCDD et PCDF substitués en 2,3,7,8. Par exemple, la DL_{50} par voie orale varie, pour les 2,3,7,8-TCDD, de 0,6 µg/kg de poids corporel chez le cobaye à 5051 µg/kg chez le hamster doré syrien. Les différences considérables de sensibilité vis-à-vis des 2,3,7,8-TCDD et des composés apparentés qu'on observe ainsi chez les diverses espèces et souches est impossible à expliquer par les différences qu'on constate dans la

toxicocinétique. La toxicité et la toxicocinétique des TCDD chez les
singes sont à peu près les mêmes que chez l'homme. Chez des souris de
lignée pure, on a des raisons de penser que la concentration
cellulaire du récepteur aux Ah est partiellement corrélée avec la
sensibilité à l'action biologique et toxique de ces composés. Le
récepteur a été retrouvé chez d'autres espèces, notamment chez
l'homme. Cependant, l'abondance relative du récepteur aux Ah selon les
espèces n'explique pas entièrement les différences de sensibilité.

1.4.2   Homme

      Malgré de nombreuses études cliniques et études de suivi
post-thérapeutiques, aucun effet général persistant bien net n'a été
mis en évidence, si ce n'est une chloracné, à la suite d'expositions
professionnelles ou accidentelles aux PCDD et aux PCDF. D'autres
effets ont été notés mais aucun n'est persistant, en dehors de la
chloracné et, peut-être, de troubles fonctionnels minimes.

      Dans quelques études épidémiologiques consacrées à des cas
d'exposition à un mélange de dioxines, de furannes et d'autres
produits chimiques, on a fait état d'une augmentation de l'incidence
des cancers de localisations diverses, mais plusieurs facteurs ne
permettent pas de faire entièrement foi à ces observations.

      Lors de l'accident de Seveso, le seul effet nocif indiscutable a
été la chloracné. Cette affection (193 cas) s'est déclarée en 1976 et
1977 et elle était encore active en 1984 chez 20 des victimes. De
nombreuses études ont été consacrées à la recherche de liens possibles
entre l'exposition à l'Agent orange et des effets nocifs chez les
civils ou les militaires, au Viet Nam. Cependant, les données dont on
dispose à l'heure actuelle ne permettent pas de conclure
catégoriquement à l'existence d'effets sur la reproduction ou d'autres
effets biologiques notables.

      Lors de l'incident survenu au Missouri, les enfants chez qui l'on
avait observé des signes aigus d'intoxication lors de la
contamination, en 1971, sont, semble-t-il, aujourd'hui en bonne santé.
En outre, des études épidémiologiques poursuivies dans ce même Etat au
sein de populations plus longuement exposées à des concentrations plus
faibles de dioxine n'ont jusqu'ici révélé aucun effet appréciable sur
la santé. Mais si aucun symptôme clinique n'a été observé, on a
constaté un effet sur l'immunité à médiation cellulaire.

      Les seuls cas d'intoxication humaine par les PCDF pour lesquels
on dispose d'observations circonstanciées concernent la contamination
d'huile de riz par des PCDF, des PCB et des PCQ, observée à Yusho au

Japon, en 1968, et Yu-cheng à Taiwan, en 1979. Au total, plusieurs milliers de personnes ont été gravement intoxiquées. Il semble très probable que les PCDF sont à incriminer dans ces intoxications. La symptomatologie générale était analogue à celle qui accompagne les intoxi-cations par les TCDD, compte tenu des différences traduisant l'intensité de l'exposition et la structure d'âge et de sexe des groupes exposés.

A Yusho, l'apport quotidien moyen de PCDF substitués en 2,3,7,8 a été estimé à 0,1-0,2 µg/kg de poids corporel sur plusieurs mois, alors que la dose pathogène minimale a été estimée à 0,05-0,1 µg/kg de poids corporel par jour, sur 30 jours.

1.5 Conclusion

Les PCDD et les PCDF se rencontrent dans tout l'environnement et il est probable que chacun en est porteur. Ces composés déterminent parfois des effets toxiques complexes en cas d'exposition professionnelle ou accidentelle.

D'après les observations faites à Yusho, les expériences conduites chez des espèces sensibles de singes, et compte tenu de certaines hypothèses concernant l'activité relative des PCDD et des PCDF, il se peut que l'homme et certains singes présentent une sensibilité comparable à l'égard de ces composés. Cependant, les incertitudes concernant la dose effectivement reçue et les difficultés de l'évaluation des effets toxiques autres que la chloracné interdisent, dans le cas de l'homme, une conclusion catégorique quant à la résistance relative de l'homme aux effets toxiques de ces composés. Il convient de limiter l'exposition aux plus faibles niveaux compatibles avec les impératifs pratiques.

2.   Recommandations

1.   Des études interlaboratoires de validation, selon une sorte de "poule" avec protocoles normalisés de contrôle et d'assurance de qualité, sont indispensables pour améliorer les méthodes d'analyse.

La méthodologie de l'échantillonnage, les méthodes d'analyse et l'interprétation des données doivent être optimalisées et normalisées avant le démarrage des enquêtes.

2.   Des données complémentaires sont nécessaires sur l'origine des PCDD et des PCDF, sur leur répartition dans l'environnement et sur leur destinée.

D'autres observations sont indispensables au sujet de la présence des PCDD ou des PCDF dans l'environnement, notamment sur l'évolution des concentrations dans le temps et sur la détermination des profils isomériques, notamment dans les produits alimentaires, dans l'air ambiant et dans les sédiments.

3.   Il serait bon de faire des observations sur les effets qu'exercent les PCDD et les PCDF sur la biocénose.

4.   Des données plus complètes sont nécessaires en ce qui concerne la biodisponibilité des PCDD et des PCDF provenant de diverses matrices, dans l'environnement et dans la ration alimentaire. L'exposition à ces sources devrait être mise en corrélation avec les pratiques agricoles et industrielles.

5.   Il convient de mettre au point et de valider des méthodes chimiques et biologiques plus simples et moins coûteuses pour rechercher la présence de PCDD et de PCDF.

6.   Des études sur les mécanismes toxiques des PCDD et des PCDF sont nécessaires pour élucider les différences observées dans les effets produits chez les diverses espèces et permettre une extrapolation à l'homme.

7.   Il importe de poursuivre l'étude de l'immunotoxicité, notamment en ce qui concerne la fonction des lymphocytes T cytotoxiques. En particulier, il est capital d'étudier la nature et la durée des effets sur le système immunitaire d'une exposition pendant la période périnatale.

8.   Des études de toxicité à long terme devraient être effectuées, notamment des études de reproduction sur plusieurs générations chez diverses espèces, au moyen de trois des PCDD et des PCDF les plus répandus, à savoir le 2,3,4,7,8-pentaCDF, le 1,2,3,7,8-pentaCDD et l'octaCDD.

9.   Etant donné que l'homme est exposé à des mélanges complexes de PCDD et de PCDF, il faut poursuivre la mise au point et la validation de divers systèmes d'épreuve, notamment des cultures de cellules humaines en vue d'apprécier l'activité toxique de ces composés et de leurs mélanges. Ces systèmes permettront d'étudier les mécanismes d'action, les relations structure/activité et les effets interactifs.

10. Il serait bon d'étudier la charge de l'organisme en PCDD et PCDF et d'établir une corrélation entre cette charge, les effets cliniques constatés et les résultats des examens de laboratoire. Le suivi de

groupes exposés antérieurement est important à cet égard.

EVALUATION DES DANGERS POUR LA SANTE DE L'HOMME
DE L'EXPOSITION AUX DIBENZO-P-DIOXINES POLYCHLORES (PCDD)
ET AUX DIBENZOFURANNES POLYCHLORES (PCDF)

1. Introduction

    Pour évaluer les dangers que représentent les PCDD et les PCDF
pour la santé humaine, il faut connaître à la fois l'intensité de
l'exposition et les effets biologiques correspondants.

    Il est fort difficile d'évaluer l'exposition humaine. Plusieurs
méthodes sont possibles, par exemple:

    a)   Utilisation de modèles physiologiques classiques de
          l'inhalation, de l'ingestion et de l'absorption cutanée. Il
          faut connaître de façon précise la concentration des
          produits en cause dans les différents milieux et dans les
          denrées alimentaires, ainsi que leur biodisponibilité.

    b)   Estimations de l'apport sur la base de modèles
          pharmacocinétiques simples et des teneurs observées dans les
          divers tissus humains en vue d'une évaluation précise de
          l'exposition. Il faut en outre connaître en détail l'apport,
          la distribution, le métabolisme et l'élimination des PCDD et
          des PCDF chez l'homme.

2.   Evaluation de l'exposition

2.1   Sources de contamination

    Les principales sources de PCDD et de PCDF identifiées jusqu'ici
sont les contaminants contenus dans les produits chimiques vendus dans
le commerce (voir section 3.3), les émissions par suite de combustion
(voir section 3.5) et l'élimination de déchets industriels contenant
des PCDD ou des PCDF (sections 3.4, 3.5.9 et 3.5.10).

    Dans certains cas, on peut estimer au niveau local la
contribution relative de ces diverses sources. Mais, les méthodes et
les données dont on dispose aujourd'hui ne permettent pas de
conclusions définitives quant à leur importance relative au niveau
d'un pays ou du monde entier.

2.2   Concentrations dans le milieu ambiant

Les données restreintes dont on dispose montrent que la
concentration atmosphérique résiduelle est très faible (fg/m^3) pour
les 2,3,7,8-tétrachloro-PCDD ou PCDF ainsi que pour les dérivés
pentachlorés ou hexachlorés. (Si l'on tient compte des analogues
heptachlorés ou octachlorés, on observe des concentrations de l'ordre
du picogramme par mètre cube).

D'après les rares données disponibles, il est peu probable qu'on
trouve des tétrachloro-2,3,7,8-PCDD ou PCDF ni des dérivés
pentachlorés ou hexachlorés dans une eau de boisson traitée, même à la
concentration de 1 pg/litre (voir section 5.2). Dans tous les
échantillons de sols et de sédiments analysés (provenant de régions
industrialisées ou non), la concentration de PCDD et de PCDF allait de
quelques nanogrammes à plusieurs centaines de nanogrammes par
kilogramme (la concentration la plus élevée concernant les sédiments
et le sol en zone urbaine).

On ne connaît pas la concentration résiduelle des PCDD et des
PCDF dans les végétaux en général.

Des concentrations atteignant 50 ng/kg ont été observées chez des
poissons capturés dans la nature pour les mêmes dérivés
(principalement les dérivés tétrachlorés et pentachlorés). Le plus
souvent, ces composés ont été observés dans des poissons gras ou des
poissons qui se nourrissent sur le fond (voir section 4.4.2).

Les observations concernant les organismes terrestres ne
permettent pas d'estimer les concentrations résiduelles (voir section
4.4.3). Dans trois échantillons d'un mélange de différents laits de
vache, on a trouvé, pour le lait entier, une concentration maximale de
100 pg/kg (voir section 5.4). La contamination des autres denrées
alimentaires vendues dans le commerce est également très mal connue.
L'analyse de plusieurs échantillons de poulet et de porc montre que
ces produits contiennent environ 5-30 ng/kg d'analogues fortement
chlorés. Le profil de ces analogues n'est pas le même que chez les
organismes aquatiques (voir section 5.4).

Les données disponibles ne permettent pas d'évaluer l'exposition
globale de la population dans son ensemble. Elles suffisent pour une
évaluation limitée de l'exposition dans le cas des populations
locales. Compte tenu des concentrations environnementales examinées
ci-dessus et des hypothèses habituelles sur l'apport d'aliments, d'air
et d'eau, les denrées alimentaires risquent davantage de contribuer
notablement à l'exposition aux PCDD/PCDF que l'air, tandis que l'eau
de boisson est sans doute beaucoup moins en cause.

2.3 Modalités d'exposition

       Chez l'homme, le tissu adipeux contient des tétrachloro-2,3,7,8
PCDD/PCDF ainsi que des dérivés pentachlorés et hexachlorés. Cette
contamination s'explique sans doute par une exposition aux produits
présents dans l'environnement, aux concentrations indiquées plus haut.

       En outre, les nourrissons peuvent être exposés par
l'intermédiaire du lait maternel et les jeunes enfants par l'ingestion
de terre contaminée. Mais, dans la plupart des cas, ce dernier mode
d'exposition ne devrait être pris en compte que dans les régions
fortement polluées.

       Certaines populations sont particulièrement exposées à l'occasion
d'accidents survenus dans l'industrie (et des opérations de nettoyage
ultérieures) lors de la production et de l'utilisation normales de
chlorophénols, d'herbicides phénoxylés et de PCB. En pareil cas, ce
sont l'inhalation et le contact cutané qui constituent les modes
d'exposition les plus importants. Cependant, on ne dispose pas de
données quantitatives sur la nature et la concentration des
contaminants.

       Sur la base des valeurs indiquées plus haut pour les
concentrations dans le milieu ambiant et des hypothèses habituelles
sur les paramètres physiologiques et les valeurs de l'apport, il est
probable que l'ingestion constitue le mode d'exposition essentiel.
L'inhalation de l'air ambiant ne devrait pas poser de problème encore
qu'un air fortement contaminé puisse contribuer sensiblement à
l'exposition. En général, il n'est pas possible actuellement d'estimer
l'importance relative de l'exposition par voie cutanée.

2.4 Biodisponibilité

       On ne dispose d'aucune donnée sur la biodisponibilité provenant
d'études sur l'homme. L'expérimentation animale montre que la
biodisponibilité des PCDD ou des PCDF après ingestion dépend de la
matrice du produit ingéré. Les données disponibles au sujet de
l'apport par voie orale sont résumées au tableau 46.

       Des études sur des rats glabres montrent que l'exposition cutanée
par contact de la peau intacte avec un sol contaminé représente
environ 1-2%. On ne dispose d'aucune donnée pour l'exposition par
inhalation.

3.   Résultats de l'exposition animale

3.1 Toxicocinétique des 2,3,7,8-TCDD

     Des études sur des rongeurs à qui l'on administrait du
2,3,7,8-TCDD par voie orale, en une ou plusieurs prises, ont montré
que l'absorption dans les voies digestives était d'au moins 50% chez
le rat, le cobaye et le hamster, mais de moins de 30% chez la souris
(tableau 40). Les valeurs indiquées pour la demi-vie d'élimination
étaient de l'ordre de 12-31 jours pour le rat, la souris et le hamster
et de 22-94 jours pour le cobaye (tableau 41). Cependant, la plupart
de ces études ont été effectuées en utilisant des doses toxiques. La
demi-vie des 2,3,7,8-TCDD chez les primates est mal connue mais il
semble, d'après les données disponibles pour le rhésus, que la
demi-vie apparente soit de l'ordre de 1 an dans les tissus adipeux.

     Les 2,3,7,8-TCDD ne s'accumulent pas dans les tissus animaux.
Chez les rongeurs, l'accumulation intervient principalement au niveau
du foie et du tissu adipeux (tableaux 42 à 44). Chez des rhésus
(tableau 45), on a observé une concentration élevée de ces mêmes
produits dans le tissu adipeux, le foie, la peau et les muscles.

     Chez des rats ayant reçu quotidiennement pendant 2 ans des
2,3,7,8-TCDD à raison de 1 ng/kg de poids corporel, on a observé une
accumulation de 540 ng/kg de poids corporel dans le foie où l'on a
également noté certaines altérations morphologiques. Des quantités du
même ordre ont été notées chez le péromysque après exposition à un sol
contenant des 2,3,7,8-TCDD à la concentration de 10-710 ng/kg
(Cockherham et al., 1980). L'exposition des animaux, au niveau de
l'ensemble de leur corps, à un sol présentant de telles teneurs peut
donc se traduire par une concentration tissulaire qui a des effets
observables chez les animaux d'expérience.

     Les TCDD sont en grande partie éliminés dans les déjections,
encore qu'il existe une certaine excrétion urinaire. Elle est plus
importante chez le hamster que chez les autres espèces étudiées.

     La transformation des 2,3,7,8-TCDD en métabolites à caractère
polaire plus prononcé s'observe chez toutes les espèces animales
étudiées (voir section 6.2 et tableau 62). L'élimination fécale et
urinaire des métabolites est rapide chez toutes ces espèces, sauf chez
le cobaye. Les métabolites connus sont beaucoup moins toxiques que les
composés dont ils dérivent.

3.2 Toxicocinétique des PCDD et des PCDF autres que les TCDD

     En dehors du cas des dérivés tétrachlorés, les observations sur
la toxicocinétique des PCDD chez les animaux sont limitées. De ce

point de vue, les PCDF ont été davantage étudiés. La demi-vie des 2,3,7,8-TCDF serait de l'ordre de 2 à 8 jours chez le rat, la souris et le rhésus et de plus de 20 jours chez le cobaye (tableau 65). Les études sur le rat montrent que les 2,3,7,8-pentaCDF se fixent davantage dans les tissus que les 2,3,7,8-TCDF (65% et 3,8% respectivement, au bout de 5 jours).

La rétention tissulaire des PCDD et des PCDF chez diverses espèces exposées à des mélanges de synthèse ou à des échantillons prélevés dans l'environnement et contenant des PCDD et des PCDF se révèle très inégale selon que les produits sont ou non substitués par des atomes de chlore dans toutes les positions 2,3,7 et 8.

3.3 Effets toxiques des 2,3,7,8-TCDD

Les effets toxiques et biologiques découlant de l'exposition aux 2,3,7,8-TCDD dépendent de divers facteurs, dont l'espèce, la souche, l'âge et le sexe des animaux d'expérience. Entre autres signes de toxicité observés chez plusieurs espèces animales, on note une perte de poids, une hépatoxicité, une porphyrie, une atteinte cutanée, des

lésions gastriques, une atrophie du thymus, une atteinte du système immunitaire, des effets tératogènes, des effets sur la reproduction et des effets cancérogènes. Les TCDD exercent des effets biologiques extrêmement divers, notamment l'induction de certaines enzymes et une carence en vitamine A. Ces effets ne sont pas tous observés chez la même espèce animale. Les effets toxiques les plus caractéristiques qu'on retrouve chez tous les animaux de laboratoire sont la perte de poids, l'atrophie du thymus et l'immunotoxicité. Chez l'homme, une chloracné et des lésions cutanées apparentées constituent les signes les plus fréquents d'une intoxication par les 2,3,7,8-TCDD; on observe également des lésions cutanées chez le rhésus, la souris glabre et le lapin. En revanche, l'exposition au 2,3,7,8-TCDD ne provoque ni chloracné ni lésions cutanées de ce type chez le rat, la plupart des souris, le cobaye et le hamster. Les lésions toxiques observées sont fréquemment soit des lésions hyperplasiques/ métaplasiques, soit des lésions hypoplasiques et elles intéressent principalement les tissus épithéliaux.

Des effets toxiques sur la reproduction ont été observés chez les singes rhésus: la plus faible dose sans effets observables serait, d'après les calculs, de 1 à 2 ng/kg de poids corporel par jour. Il se peut qu'il existe une dose sans effets observables de 1 ng/kg de poids corporel par jour chez le rat, s'agissant des effets sur la reproduction (Murray et al., 1979; Nisbet & Paxton, 1982).

Lorsqu'on compare les études consacrées à la production de cancers chez le rat par Kociba et al. (1978) et par le NTP (1982a, b), il apparaît clairement que les tumeurs hépatiques, notamment les épithéliomas hépatocellulaires, s'obtiennent à des doses similaires. Bien que le NTP ainsi que Kociba et al. (1978) aient observé une augmentation de l'incidence des tumeurs au niveau d'autres organes, les résultats ne sont pas analogues dans les deux études. Cela tient peut-être, en partie, à des différences dans les doses administrées (gavage ou exposition par incorporation à des aliments broyés) et dans les souches. Dans l'étude de Kociba, l'administration d'une dose de 10 ng/kg de poids corporel a provoqué une augmentation de l'incidence des nodules néoplasiques (hyperplasiques) chez les femelles, tandis qu'une dose de 1 ng/kg de poids corporel se traduisait par un gonflement des hépatocytes en foyers ou par places. A ces doses, l'incidence de certaines tumeurs à déterminisme hormonal était plus faible chez les animaux d'expérience que chez les témoins, ce qui donne à penser que les 2,3,7,8-TCDD déterminent des altérations au niveau des glandes endocrines. Compte tenu de ces résultats de l'expérimentation animale et des données dont on dispose au sujet de l'homme, le CIRC (1982, suppl. 4) est arrivé à la conclusion que les preuves de cancérogénicité des TCDD étaient suffisantes chez les animaux, mais insuffisantes chez l'homme.

Apparemment, les TCDD n'ont pas de propriétés mutagènes et ne devraient donc pas être génotoxiques. Leur action cancérogène est donc attribuée à un mécanisme indirect (épigénétique).

3.4 Effets toxiques des PCDD et des PCDF autres que les TCDD

Plusieurs autres PCDD et PCDF provoquent les mêmes symptômes que les 2,3,7,8-TCDD, mais leur activité est très inégale (tableaux 56, 62). Pour résumer, il existe 12 isomères qui présentent une toxicité élevée, à savoir les tétra-, penta-, hexa- et heptaCDD et CDF contenant quatre atomes de chlore symétriques deux à deux en position 2,3,7 et 8. On a montré, lors d'études prolongées sur l'animal, qu'un mélange de deux hexaCDD (1,2,3,7,8-9 et 1,2,3,6,7,8-haxaCDD) était cancérogène, mais à des doses plus élevées que celles utilisées dans l'étude des TCDD. La dioxine non substituée et la dichloro-2,7 dioxine n'ont manifesté aucun pouvoir cancérogène.

L'activité relative, toxique et biologique, des PCDD et des PCDF a été estimée au moyen d'études de courte durée chez le rat et d'études en culture de cellules mammaliennes. Comme critères, on a retenu l'inhibition de la prise de poids, l'atrophie du thymus, l'induction d'enzymes, la térato-génicité, la production d'acné et la kératinisation. En l'absence d'observations sur la toxicité à long

terme, les résultats fournis par ces épreuves de durée limitée
constituent actuellement la seule source qui permette un classement
par toxicité en vue de l'évaluation des dangers pour la santé de
l'homme.

Les études consacrées à des mélanges de ces composés ont mis en
évidence une synergie additive tout au plus.

3.5 Etude des différences interspécifiques

La sensibilité aux effets biologiques et toxiques des
2,3,7,8-TCDD est très inégale selon les espèces. Par exemple, la
DL_{50} par voie orale varie de 0,6 µg/kg de poids corporel chez le
cobaye à 5051 µg/kg de poids corporel chez le hamster doré syrien
(tableau 47); en outre, on a signalé des différences prononcées de
DL_{50} chez différentes souches de la même espèce (par exemple le rat
et la souris). La toxicité et la toxicocinétique des TCDD sont très
analogues chez les singes et chez l'homme. Cependant, il est
impossible d'expliquer les différences considérables de sensibilité,
selon le sexe et la souche, aux 2,3,7,8-TCDD et aux composés analogues
par les différences qu'on observe sur le plan toxicocinétique. Les
observations montrent que, chez des souris de lignée pure, l'abondance
cellulaire du récepteur Ah est partiellement corrélée avec la
sensibilité aux effets biologiques et toxiques de ces composés. On a
également repéré ce récepteur chez d'autres espèces, notamment chez
l'homme. Cependant, l'abondance relative de ce récepteur selon les
espèces n'explique pas les différences de sensibilité aux
2,3,7,8-TCDD; cette constatation n'a rien d'étonnant quand on sait
qu'il existe des mécanismes de toxicité complexes et encore inconnus
qui font intervenir de multiples facteurs en plus du récepteur aux Ah.

4. Effets sur la santé de l'homme

4.1 PCDD

La population générale est exposée à de faibles quantités de PCDD
et de PCDF présentes dans des mélanges complexes qui ne provoquent
aucun cas de maladie. Lors de quelques incidents, par exemple à Seveso
et à Yusho, des ouvriers et d'autres personnes sont tombés malades à
la suite d'une exposition à des quantités plus élevées d'un nombre
restreint de ces composés.

Dans le cas de l'exposition professionnelle ou accidentelle,
l'effet clinique le plus net réside dans une chloracné. D'autres
effets (tableau 64) ont été observés mais, à part la chloracné et

peut-être des troubles fonctionnels mineurs, aucun n'a été durable.

Dans certaines des études de mortalité réalisées, on a fait état d'une augmentation de l'incidence des cancers de diverses localisations, mais le trop petit nombre de cas incite à la prudence.

L'impression générale qui se dégage des études de suivi est que les effets généraux aigus des TCDD, même lorsqu'ils sont graves, sont habituellement réversibles, à l'exception de la chloracné, ou s'améliorent peu à peu après l'arrêt de l'exposition. A Seveso, le seul effet nocif sur la santé qui soit indiscutable a été la chloracné: sur les 193 cas qui se sont déclarés en 1976 et 1977, 20 présentaient encore des symptômes actifs en 1984. De nombreuses études ont été consacrées à la recherche de liens possibles entre l'exposition à l'Agent orange, au Viet Nam, et divers effets sur la santé, tant chez les civils que chez les militaires. Cependant, les données dont on dispose à ce jour ne permettent pas de conclure catégoriquement à l'existence d'effets biologiques notables, en particulier sur la reproduction (voir section 9.2).

Dans un certain nombre d'études, on a comparé les populations exposées à divers groupes témoins sur la base du dosage des lipides sériques, d'épreuves de la fonction hépatique et de divers autres paramètres. Bien que dans ces études ainsi que dans des études de cas isolés, on ait signalé des différences statistiquement significatives, il est impossible de formuler des conclusions définitives par suite du manque d'uniformité, de diverses insuffisances sur le plan technique et de l'impossibilité d'exclure les facteurs de confusion.

Lors de la contamination accidentelle survenue en 1971 au Missouri (Etats-Unis d'Amérique), des enfants ont manifesté des signes d'intoxication aiguë mais ils sont aujourd'hui en bonne santé, semble-t-il. Les études épidémiologiques conduites dans le même Etat sur des populations durablement exposées à des concentrations plus faibles n'ont jusqu'ici révélé aucun effet biologique notable.

Cependant, malgré l'absence de manifestations cliniques, certains signes témoignent d'une action sur le système immunitaire à médiation cellulaire.

La gamme des effets des TCDD sur la santé humaine reste à préciser. On peut conclure que, dans l'ensemble, les observations concernant l'exposition et les effets sur la santé ne permettent pas d'établir pour l'homme des relations dose/effet ou dose/réponse.

En dépit de nombreuses études cliniques et études de suivi, aucun

effet général persistant bien net n'a été observé, à l'exception de la chloracné. Compte tenu de ce qu'on sait actuellement, il semble peu probable que l'exposition aux TCDD ait des séquelles toxicologiques permanentes, graves et invalidantes.

4.2 PCDF

Les seuls cas d'intoxication humaine par les PCDF pour lesquels on dispose d'informations circonstanciées concernent les incidents survenus à Yusho, au Japon (1968) et à Yu-cheng, à Taiwan (1979), à la suite d'une contamination d'huile de riz par des PCDF, des PCD et des PCQ (voir section 11.1). Au total, plusieurs milliers de personnes ont été gravement intoxiquées. La récapitulation des données montre que, selon toute vraisemblance, il faut incriminer les PCDF. La symptomatologie générale était sensiblement la même que lors d'une intoxication par les PCDD. Les différences reflètent peut-être l'intensité de l'exposition et la structure, par âge et sexe, de la population exposée. Dans le cas des malades de Yusho, les estimations de l'apport quotidien moyen de PCDF pendant plusieurs mois ont abouti à une dose de 0,9 µg/kg de poids corporel pour les PCDF totaux, de 0,1-0,2 µg/kg pour les tétrachloro-2,3,7,8 dibenzofurannes ainsi que les penta- et hexaPCDF, à quoi il faut ajouter 150 µg pour les PCB et 148 µg pour les PCQ par kg de poids corporel (Hayabuchi et al., 1979). La dose pathogène minimale a été estimée à 0,6 mg de PCDF totaux par personne sur 30 jours, ce qui correspond à une dose quotidienne de 0,05-0,1 µg/kg de poids corporel pour les PCDF substitués en 2,3,7 et 8. Quoi qu'il en soit, les données disponibles sont insuffisantes pour qu'on puisse se prononcer sur la valeur de la dose qui serait sans danger pour l'homme.

4.3 Charge de l'organisme humain et cinétique

Chez l'homme, la concentration résiduelle des TCDD dans le tissu adipeux s'élève jusqu'à 20 mg/kg dans la population générale, en dehors de toute exposition précise connue, mais elle peut atteindre une valeur plus élevée dans certains cas sans entraîner de manifestations pathologiques. Aucune des populations ainsi étudiées n'avait été constituée par sondage aléatoire. Les autres PCDD et PCDF contenant un plus grand nombre d'atomes de chlore, en particulier les octaDD, se rencontrent également dans ces échantillons (voir tableaux 29, 30). Les concentrations moyennes semblent augmenter avec l'âge.

Dans certaines situations, on a observé des concen-trations plus élevées (de l'ordre de quelques microgrammes par kilogramme) qui ne s'accompagnaient d'aucune manifestation pathologique.

Lors des accidents de Yusho et de Yu-cheng, les symptômes observés étaient associés à des concentrations de PCDF plus élevées; par exemple, on a trouvé, un an après exposition à de l'huile de riz contaminée, une concentration de 6,9 µg/kg de tissu adipeux pour les pentachloro-2,3,4,7,8 dibenzofurannes.

D'après les données très limitées dont on dispose, la concentration dans la population générale du 2,3,4,7,8- pentaCDF, par exemple, semble cent fois plus faible que la concentration observée chez les victimes de l'accident de Yusho.

Aucune comparaison de ce type n'est possible pour les PCDD.

Des données limitées montrent que, à l'exception des TCDF, les isomères chlorés en position 2,3,7 et 8 font l'objet d'une rétention sélective.

D'après une étude expérimentale unique, la demi-vie des TCDD serait de 5 ans chez l'homme. Une autre étude a permis d'évaluer cette demi-vie à 2-6 ans pour les 1,2,3,6,7,8- hexaCDD, les 1,2,3,4,6,7,8-heptaCDD, les octaCDD, les 1,2,3,4,6,7,8-heptaCDF et les octaCDF. Ces observations devront être complétées car elles proviennent d'études qui portaient sur deux sujets seulement, sans compter que la toxicinétique de ces types de composés ne se ramène peut-être pas simplement à une cinétique du premier ordre. Cependant, même si les données actuelles ont des limites, il est clair que la demi-vie de ces composés est de l'ordre d'une ou plusieurs années.

Les valeurs indiquées pour la demi-vie sont très différentes chez l'homme et chez les rongeurs. Mais, les expériences conduites sur les animaux ont généralement été exécutées au moyen de doses toxiques. En outre, les animaux qui ont une moindre longévité ont un métabolisme plus important de sorte qu'il est normal que la demi-vie soit plus courte.

Les PCDD et les PCDF sont essentiellement retenus dans les graisses, mais ces produits sont aussi excrétés dans le lait (tableau 39) et traversent la barrière placentaire. On les trouve aussi, à plus faibles concentrations, dans le sang et les organes vitaux. La distribution entre les différents tissus humains est mal connue mais selon certains, le rapport des concentrations dans le tissu adipeux et dans le foie est plus élevé chez l'homme que chez les rongeurs. Cependant, cette conclusion repose sur des données fort restreintes fondées sur des pièces d'autopsie. Il reste donc à voir si elle vaut pour la population dans son ensemble.

Les voies de pénétration dans l'organisme humain sont encore mal connues mais on admet que l'apport alimentaire représente l'essentiel. Cependant, le nourrisson représente un cas particulier puisque, par suite du passage trans-placentaire, il peut y avoir une exposition in utero. D'après les dosages effectués jusqu'ici dans le lait maternel, ce dernier pourrait être une source importante de contamination.

Il n'existe aucune donnée concernant la dose des PCDD qui est toxique pour l'homme. Toutefois, lors des accidents de Yusho et de Yu-cheng, les victimes avaient absorbé une dose totale de l'ordre de 3,3-3,8 mg pour les PCDF totaux et de 400-500 µg pour les PCDF substitués en 2,3,7 et 8. Il n'existe aucune donnée fiable sur les doses sans effet nocif.

5. Conclusions générales

Les PCDD et les PCDF se rencontrent dans tout l'environnement et il est probable que chacun en est porteur. Parfois, ces produits déterminent des effets toxiques complexes à la suite d'une activité professionnelle ou accidentelle.

D'après les observations faites à Yusho, les expériences conduites chez des espèces sensibles de singes et compte tenu de certaines hypothèses concernant l'activité relative des PCDD et des PCDF, il se peut que l'homme et certains singes présentent une sensibilité comparable à l'égard de ces composés. Cependant, les incertitudes concernant la dose effectivement reçue et les difficultés de l'évaluation des effets toxiques autres que la chloracné interdisent, dans le cas de l'homme, une conclusion catégorique quant à la résistance relative de l'homme aux effets toxiques de ces composés. Il convient de limiter l'exposition aux plus faibles niveaux compatibles avec les impératifs pratiques.

RECOMMANDATIONS

1. Des études interlaboratoires de validation, selon une sorte de "poule" et l'utilisation de protocoles normalisés de contrôle et d'assurance de qualité, sont indispensables pour améliorer les méthodes d'analyse.

    La méthodologie de l'échantillonnage, les méthodes d'analyse et l'interprétation des données doivent être optimalisées et normalisées avant le démarrage des enquêtes.

2. Des données complémentaires sont nécessaires sur l'origine des

PCDD et des PCDF, sur leur répartition dans l'environnement et
sur leur destinée.

D'autres observations sont indispensables au sujet de la présence
des PCDD ou des PCDF dans l'environnement, notamment sur
l'évolution des concentrations dans le temps et sur la
détermination des profils isomériques, notamment dans les
produits alimentaires, dans l'air ambiant et dans les sédiments.

3. Il serait bon de faire des observations sur les effets
   qu'exercent les PCDD et les PCDF sur la biocénose.

4. Des données plus complètes sont nécessaires en ce qui concerne la
   biodisponibilité des PCDD et des PCDF provenant de diverses
   matrices, dans l'environnement et dans la ration alimentaire.
   L'exposition à ces sources devrait être mise en corrélation avec
   les pratiques agricoles et industrielles.

5. Il convient de mettre au point et de valider des méthodes
   chimiques et biologiques plus simples et moins coûteuses pour
   rechercher la présence de PCDD et de PCDF.

6. Des études sur les mécanismes toxiques des PCDD et des PCDF sont
   nécessaires pour élucider les différences observées dans les
   effets produits chez les diverses espèces et permettre une
   extrapolation à l'homme.

7. Il importe de poursuivre l'étude de l'immunotoxicité, notamment
   en ce qui concerne la fonction des lymphocytes T cytotoxiques. En
   particulier, il est capital d'étudier les effets sur le système
   immunitaire d'une exposition à la période périnatale et de la
   durée de cette action.

8. Des études de toxicité à long terme devraient être effectuées,
   notamment des études de reproduction sur plusieurs générations
   chez diverses espèces, au moyen de trois des PCDD et des PCDF les
   plus répandus, à savoir le 2,3,4,7,8-pentaCDF, le
   1,2,3,7,8-pentaCDD et l'octaCDD.

9. Etant donné que l'homme est exposé à des mélanges complexes de
   PCDD et de PCDF, il faut poursuivre la mise au point et la
   validation de systèmes d'épreuve, permmettant notamment l'étude
   des réactions des tissus humains en vue d'apprécier l'activité
   toxique de ces composés et de leurs mélanges. Ces systèmes
   permettront d'étudier les mécanismes d'action, les relations
   structure/activité et les effets interactifs.

10.   Il serait bon d'étudier la charge de l'organisme en PCDD et PCDF
      et d'établir une corrélation entre cette charge, les effets
      cliniques constatés et les résultats des examens de laboratoire.
      Le suivi des groupes exposés antérieurement est important à cet
      égard.

See Also:
        [Toxicological Abbreviations](#)