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Description Notes Transmitted to Alvin L, Young from Wendy, U.S. Environmental Protection Agency, Office of International Activities, Associate Administrator, dated February 26, 1986

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF INTERNATIONAL ACTIVITIES
ASSOCIATE ADMINISTRATOR

February 26, 1986

Al,

Here is a draft of the WHO/IPCS report on dioxin.

Wendy

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 21 1986

OFFICE OF
INTERNATIONAL ACTIVITIES

MEMORANDUM

TO: Scott Baker, Special Assistant to the AA for ORD
Peter Preuss, Acting Director, OHEA
Jack Fowle, OHR
Joseph Merenda, Director, OTS/ECAD
Carole Gray, OPP
Walter Kovalick, Deputy Director, OERR
Edwin Johnson, Chief of Staff, OW
Joseph Cotruvo, Director, ODW/CSD

FROM: Pete Christich P.C.

SUBJECT: Agency Review and Comment on draft UN Criteria Document on Dioxins and Dibenzofurans

Dr. Mercier, Manager of IPCS, has asked EPA to review and provide written comments on the attached draft IPCS environmental health criteria document; see the attached February 10 letter on this request. Please provide your office's written comments to me no later than April 15, 1986. Dr. Mercier has a comment deadline of May 15, 1986.

After I receive each office's comments, I will circulate the entire EPA comments package to EPA contacts for IPCS who want to see them for peer review. I want to send the peer-reviewed Agency comment package to Dr. Mercier during the first week of May.

Call me on 382-4893 or 382-4895 if you want to receive the EPA comment package prior to my sending it to IPCS. Also, advise me if any additional staff should be given a copy of the draft IPCS criteria document for review.

Attachment

cc: (with draft criteria document)
Lester Grant, ECAO/RTP
Jerry Stara, ECAO/Cincinnati
Donald Barnes, OPTS
Breck Milroy, OTS
Larry Rosenstein, OTS
Barry Korb, OSWER

WORLD HEALTH ORGANIZATION ORGANISATION MONDIALE DE LA SANTE

Téléphone Central/Exchange: 91 21 11
Direct: 91 35 88

To all national contact points
for the Environmental Health Criteria

In reply please refer to: ICS-C18/445/3(23)
Priere de rappeler la référence:

10 February 1986

Dear Colleague,

International Programme on Chemical Safety (IPCS)
Environmental Health Criteria: Poly-Chlorinated
Dibenzo-Para-Dioxins and Dibenzofurans

I have pleasure in enclosing, for review and comment, the draft of the Environmental Health Criteria for Poly-Chlorinated Dibenzo-para-Dioxins and Dibenzofurans. When you have perused this draft, I should be pleased if you would let me know:

1. whether any significant work has been omitted; if so, please indicate the sections and chapters where you would like to see this work mentioned;
2. whether you feel that any reference mentioned in the text should be deleted giving your reasons why it is not pertinent or valid;
3. whether you have any general comments on the structure, content or length of the various chapters, or whether there are any other changes you would like to see introduced.

In connexion with (1) above, if you suggest any additional reference(s), it is essential to let us have a reprint or photocopy of each article in question (or of the relevant pages, in the case of a book).

Any comments on the text should reach me by 15 May 1986.

With many thanks for your help.

Yours sincerely,

Dr M. Mercier
Manager
International Programme on
Chemical Safety
Division of Environmental Health

ENCL: as stated

UNITED NATIONS ENVIRONMENT PROGRAMME
INTERNATIONAL LABOUR ORGANIZATION
WORLD HEALTH ORGANIZATION

ICS/85.2
UNEDITED DRAFT

IPCS ENVIRONMENTAL HEALTH CRITERIA
POLY-CHLORINATED DIBENZO-PARA-DIOXINS AND DIBENZOFURANS

I P C S

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

ENVIRONMENTAL HEALTH CRITERIA: POLY-CHLORINATED DIBENZO-PARA-DIOXINS AND DIBENZOFURANS

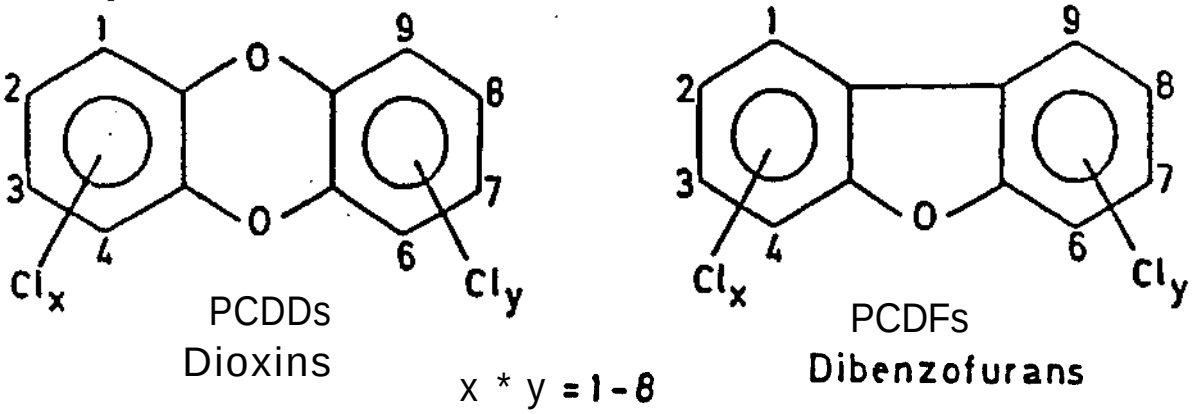
Contents page

1. SUMMARY AND RECOMMENDATIONS FOR FURTHER RESEARCH
(to be elaborated by the Task Group)

2. CHEMICAL AND PHYSICAL DATA

The polychlorinated dibenzo-para-dioxins (PCODs) and polychlorinated dibenzofurans (PCDFs) are two series of almost planar tricyclic aromatic compounds with very similar chemical properties. The general formulaes are given in Fig. 2-1.

Figure 2-1.



The number of chlorine atoms can vary between 1 and 8. 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 are given in Table 2-1.

Table 2-1

	PCDDs	PCDFs
Cl_1	2	4
Cl_2	10	16
Cl_3	14	28
Cl_4	22	38
Cl_5	14	28
Cl_6	10	16
Cl_7	2	4
Cl_8	1	1
	75	755

A large number of the individual PCDDs have been synthesized by various methods and characterized mainly by gas chromatography-mass spectrometry (Buser and Rappe, 1980; Buser and Rappe, 1984; Taylor et al., 1984; Rappe et al., 1984),

but also using NMR, UV, IR (Pohland and Yang, 1972; Kende et al., 1974) or X-ray analyses (Boer et al., 1972; Slonecker et al., 1983).

Taylor et al. (1985) have synthesized, separated and Isolated aU the 22 tetra-CDD isomers. In Table 2-2 are listed some other isomers, which have been synthesized and isolated.

PCDD Isomer	Synthetic method	M.P. °C	Reference
1-Chloro-	a	80-90	A
2-Chloro-	a	88-89	A
1,3-Dichloro-	a	113.5-114.5	B
2,3-Dichloro-	a	163-164	A
2,7-Dichloro-	b	209-210	A
2,8-Dichloro-	c	150.5-151	A
1,2,4-Trichloro-	d	128-129	A
2,3,7-Trichloro-	a	157-158	B
2,3,7,8-Tetrachloro-	b	305-306	A
-"-	e	305-307	B
1,2,3,4-Tetrachloro-	d	188-190	A
1,3,7,8-Tetrachloro-	a	193.5-195	B
1,3,6,8-Tetrachloro-	b	219-219.5	A
1,2,3,4,7-Pentachloro-	e	195-196	A
1,2,3,4,7,8-Hexachloro-	e	275	A
1,2,4,6,7,9-Hexachloro-	b	238-240	A
Octachloro	b	330	A

a) Catechol + chlorobenzene
b) Pyrolysis of chlorphenols
c) Cyclization of chlorophenoxyphenol
d) Catechol + chloronitrobenzene
e) Chlorination of chlorodibenzodioxin

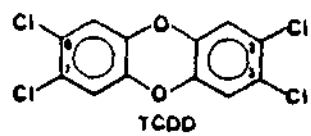
A) Pohland and Yang, 1972
B) Kende et al., 1974

The native 2,3,7,8-tetra-CDD is for sale as well as the $^{13}C_{12}$, $^{37}Cl_4$, ^{14}C and ^{3}H labelled compounds.

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

In the PCDF series Mazer et al. (1983) have synthesized all the 38 positional tetra-CDF isomers. The products were mixtures of isomers, and each of these isomers could be identified. Later Bell and GarS (1985) have isolated and characterized all the 38 tetra-CDFs, 28 penta-CDFs and 16 hexa-CDFs.

The most toxic and most extensively studied representative of the chlorinated dioxins (PCDDs) is 2,3,7,8-tetrachlorodibenzo-para-dioxins (2,3,7,8-tetra-CDD) see Figure 2-2.



Figur 2-2. 2,3,7,8-TCDD

The empirical formulae of 2,3,7,8-tetra-CDD is $C_{12}H_4Cl_4O_2$ and it has a molecular weight of 322. The melting point is 305-306°C (Pohland and Yang, 1972). Although tetra-CDD is lipophilic, it is only slightly soluble in most solvents and very slightly soluble in water (Table 2-3).

Table 2-3. Solubility of 2,3,7,8-tetra-CDD in various solvents at 25°C (Crummett and Stehl, 1973).

Solvent	Solubility g/l	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[a)]	<0.1	<0.1
Methanol	0.01	0.01
Water	0.0000002	0.0000002

a) DMSO caused detector fouling and a better value could not be obtained. However, solubility does not exceed 0.01 percent.

The vapor pressure of 2,3,7,8-tetra-CDD has been studied by Schroy et al 1984. They found that vapor of this compound behaves as an ideal gas and is valatile even at ambient soil temperatures. They give a boiling point of 421.2°C and they report the heat of sublimation for 2,3,7,8-tetra-CDD to 29637 kcal/mole.

2,3,7,8-Tetra-CDD 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 and Wong, 1977; Gebefugi ct al., 1977; Åkermark, 1978). Thermally it is very stable and rapid decomposition of 2,3,7,8-tetra-CDD occurs only at temperatures above 750°C (Stehl et al., 1973).

The first synthesis of 2,3,7,8-tetra-CDD was reported by Sandermann et al. (1957) by 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., 1971; Langer et al., 1973).

3. ANALYTICAL METHODS

3.1 General aspects

The earliest reported method used to detect 2,3,7,8-tetra-CDD 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 and 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 unspecific and not sufficiently sensitive. In the late 1960s and early 1970s chemical analytical methods were developed.

For the laboratory identification of 2,3,7,8-tetra-CDD a number of spectroscopic methods are available 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 are given by Baughman (1974), Pohland and Yang (1972) and Slonecker et al. (1983).

Because of the extreme toxicity of some PCDD and PCDF isomers, highly sensitive and specific analytical techniques are required for the measurements. Detection levels in ecological and human samples should be orders of magnitude below the usual detection levels obtained in pesticide analysis. A detection level of 1 pg (10^{-12}g) or less might be required to find 2,3,7,8-tetra-CDD and the other toxic isomers in a 1-g sample, 1 part per trillion (1 ppt) or sub-ppt. Analyses at such low levels are complicated by the presence of a multitude of other interfering compounds.

It should be mentioned that the level of sophistication applied in these analyses and the objectives thereof varied widely. In some cases the objectives have been primarily to screen samples in qualitative or semiquantitative manner, to identify groups of PCDDs and/or PCDFs. In other instances, the objective of the analysis has been to accurately quantify specific PCDD and/or PCDF isomers in the samples. Clearly, both types of analyses can be useful, depending on the purpose for which the analytical results are to be used.

In recent years, many analytical methods have been developed for the analysis of trace amounts of PCDDs and PCDFs in environmental samples, especially for the most toxic 2,3,7,8-tetra-CDD. The most specific of these methods are based on mass spectroscopy (MS). There are several requirements to be met by such an analyticalmethod:

- representative sampling and good storage
- efficient extraction
- high selectivity in the clean-up
- high specificity in the gas chromatography
- high sensitivity in the detection
- safe quantification
- good reproducibility
- useful confirmatory information

Among the many types of samples analyzed for PCDDs and PCDFs are

- technical samples like chlorphenoxy acetic acids, chlorphenols and PCB
- wastes including hazardous waste sites samples: complex chemical mixtures, landfill, sludges and leachate
- soils and sediments
- industrial hygine samples: wipes and air samples (solid sorbents)
- combustion products: fly ash, stack effluents, particulates, slag, condensate water and other process fluids from combustion of municipal waste, chemical waste, PCB, chlorphenol treated materials and wastes, wood and coal

- water: rivers, streams, lakes, wells and leachate
- animal tissues: adipose, muscle, liver, blood and whole animal (bovine, elk, deer, rat, rabbit, birds, turtle and fish)
- human tissues: adipose, liver, kidney and blood
- human and animal milk

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

3.2 Sampling methods

The procedure used in obtaining a sample to be analyzed for PCDDs/PCDFs can affect markedly the results obtained. However, it seems that this aspect is one of the most neglected areas. A detailed discussion of sampling requirements and guidelines for use in environmental chemical measurements has recently been prepared by American Chemical Society's Subcommettee on Environmental Analytical Chemistry (1983).

3.3 Extraction procedures

Many different procedures for the extraction of PCDDs/PCOFs 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), other methods are discussed by Tiernan (1983).

An interlaboratory round robin study where 13 laboratories were involved has been carried out to estimate the reliability of data on 2,3,7,8-tetra-CDD in fish. No significant differences were found from methods differing in the digestion or extraction (Ryan et al 1983).
On the contrary Lustenhouwer et al (1980) reported a dramatic difference between various solvents in the extraction efficiencies of PCDDs and PCDFs from the same sample of a fly ash.

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 vary for different sample matrices. Two different procedureal trends can be recognized

- All PCDD and PCOF isomers can be analyzed in one single fraction by the containment enrichment procedure (Stalling et al., 1983; Norstrom et al., 1982; Tiernan et al., 1983; Rappe et al., 1984).
- Specific isomers are analyzed in different fractions mainly after normal-phase and reverse-phase high pressure liquid chromatography (HPLC) separation (Lamparski et al., 1979; Tosine, 1983; Niemann et al., 1983).

This latter method allows the identification of only a few PCDD isomers, mainly 2,3,7,8-tetra-CDD. However, for a monitoring program a broader, more general method is preferred.

The method described by Stalling et al. (1983) was originally designed for the analyses of fish samples. In a round robin study it gave good results (Ryan et al., 1983). This method has now been used for the clean-up of other biological samples lika bird muscle, seal fat, turtle fat, human adipose tissue, human liver and kidney and mother's milk (Rappe et al., 1983; Rappe et al., 1985; Rappe, 1985).

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 MS instrument, which operates either in the electron impact (EI) or the negative 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 the highest toxicity, to be identified. For an unambiquous isomer identification it is necessary to have access to all isomeric standards within a specific group of congeners, e.g. all the 22 tetra-CDDs and all the 38 tetra-CDFs. All the 22 tetra-CDD have been prepared, and using a Silar 10 c glass capillary column the highly toxic 2,3,7,8-tetra-CDD can be separated from all the other 21 tetra isomers (Buser and Rappe, 1980). Recently all the 14 penta-CDDs and the 10 hexa-CDDs have been prepared. Using

the Silar 10 c column the toxic 2,3,7,8-substituted isomers can be separated from all the other isomers (Buser and Rappe, 1984). The SP 2330 fused silica column can also be used for this separation (Rappe et al, 1984) and figure 3.1 illustrates the separation of tetra-, penta- and hexa-CDDs.

In the PCDF series Mazer et al (1983) have synthesized all the 38 positional tetra-CDF isomers. The products were mixtures of isomers, and each of these isomers could be identified using both a SP 2330 and a SE 54 capillary column. Later, Bell and Garß (1984) have isolated and characterized all tetra-, penta-and hexa-CDFs. The SP 2330 column can separate most of these isomers (Rappe et al , 1984) see also Figure 3.2. The toxic 1,2,3,7,8-penta-CDF co-elutes with the 1,2,3,4,8-isomer and the toxic 1,2,3,4,7,8-hexa-CDF with the 1,2,3,4,7,9-isomer, but these isomers can be separated on less polar columns like OV-17 and DB-5.

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

3.6 Quantification

Mass specific detection (mass fragmentography) has been used to quantify trace amounts of PCDDs and PCDFs in the samples by selective monitoring M, M ± 2 and/or M ± 4 ions. The quantification is based on peak area measurements and 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 tetra-CDF isomers has shown a three-fold variation in response factors with the EI mode and up to a 20-fold variation with the NCI mode. For the higher chlorinated homologues (Cl_5, Cl_6) the difference was found to be smaller (Rappe et al , 1983).

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, 1985).
(a) Isomer specificity must be demonstrated initially and verified daily.

(b) The M/M±2 ratio must be within ± 10%.
(c> The signal to noise ratio must be 2.5:1 or better.

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. The typical fragmentation is M-COCl$^+$. Buser and 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-tetra-CDD has a 4:0 pattern. However, these low mass ions may not be observed in spectra from environmental or biological samples.

In NCI the PCDFs have the base peak due to M~ and fragmentation is the unusual M^- 34 ions (uptake of H and loss of CD. Fragmentation of PCDDs in NCI is more conventional via loss of Cl yieldings M^- 35 ions (Buser et al , 1984).

For a survey of interfering compounds see Buser, 1978 and Buser et al , 1985). Using EI technique the detection limits are 1-10 pg for the tetrachloro compounds and up to 10-50 pg for the octachloro compounds using single or multiple ion monitoring (SID or MID). Full mass spectra require 0.1-1 ng (Buser et al , 1984).

NCI using methane as reagent gas shows extremely good sensitivity for all PCDFs (tetra- to octachloro compounds) and for the higher chlorinated PCDDs (penta- to octa-CDD, detection limits are in the 10-100 fg (10^{-15} g> range using SID or MID, 1- to 2 orders of magnitude better than EI (Buser et al , 1984). However, NCI has very poor sensitivity for 2,3,7,8-tetra-CDD under these conditions.

3.8 Other analytical methods

Paasivirta et al (1977) have shown that 2,3,7,8-tetra-CDD 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, SID).

Other techniques such as enzyme induction and radioimmunoassay are described and discussed by Firestone (1978) and McKinney (1978). McKinney et al (1982) have used the radioimmunoassay method for determining 2,3,7,8-tetra-CDD in human fat and found a reliable sensitivity at 95% confidence interval to be 100 pg.

An analytical method based on the keratonization response in an in vitro system has been described by Gierthy and Crane (1985). This method can be an assay for dioxin-like activity in environmental and biological samples. A positive response was found for a 10^{-11} M solution of 2,3,7,8-tetra-CDD.

3.9 Historical aspects

In the late 1960s and early 1970s gaschromatographic methods were used for the quantification of mainly 2,3,7,8-tetra-CDD in commercial 2,4,5-T formations. The detection level was normally in the range µg/g and these analyses were not isomer specific and the results could not be confirmed.

During the 1970s and 1980s various types of mass spectrometer and gas chromatographs - mass spectrometers were in the analytical work. Using these more sophisticated instrument combinations allowed isomer specific and validated analyses for the tetra-CDDs in the very late 1970s and for the other PCDDs and PCDFs in the early 1980s.

4. SOURCES OF ENVIRONMENTAL POLLUTION

4.1 Production, synthesis and use

Polychlorinated dioxins and dibenzofurans are not commercially produced. Rather these compounds are formed as trace amounts of unwanted impurities in the manufacture of other chemicals like chlorinated phenols and their derivatives, chlorinated diphenyl ethers and polychlorinated biphenyls (PCBs). There is no known technical use for the polychlorinated dioxins, although octachloro dioxin has been described in a patent (Mulka, 1968).

It has been estimated that the total amount of 2,3,7,8-TCDD entering the US environment is 40 kg/year (SAI, 1980). The amount of total polychlorinated dioxins entering the Canadian environment/year has been speculated to be about

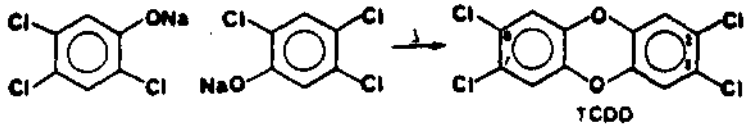
1500 kg and 75% of this amount has been estimated to be due to octa - COD alone (NRCC, 1981). There is no estimation of the amount of PCDFs entering the environment anywhere in the world.

Although the polychlorinated dioxins and dibenzofurans are not commercially produced, most of these compounds have been synthesised mostly in very minute quantitities according to the reactions discussed in section 2.

4.2 Chemical processes in industry

In addition to the synthetic methods mentioned in section 2, 2,3,7,8-tetra-CDD may also be formed during the industrial preparation of 2,4,5-trichlorophenol from 1,2,4,5-tetrachlorobenzene, Figure 4-1.

Figure 4-1



This reaction takes place at about 180°C and, when the solvent is methanol, the pressure rises to about 7KPa. The formation of TCDO 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 as above 180°C and proceed rapidly and uncontrollably to result in the formation of TCDO. It has been suggested that this reaction sequence caused the accident at Bolsover, UK (Milnes 1971). It has also been suggested that in the accident at Seveso, Italy, this series of reactions began when part of the ethylene glycol had been distilled off with the alkaline solution at 170°C, i.e. at a stage during which there was considerable risk of the occurrence of exothermic reactions (Rappe 1978).

After most of the solvent has been distilled off, the reaction mixture is acidified; the 2,4,5-trichlorophenol can be freed from 2,3,7,8-tetra-CDD by one OP two distillations, with the result that 2,3,7,8-tetra-CDD is concentrated in the residues. An level of 1000 µg (0.1%) of 2,3,7,8-tetra-CDD in such residues have been reported (Kimbrough et al, 1984). Episodes involving the improper disposal of such residues are discussed in section 4.4.2.

The main part 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, Fig 4-2) and the bactericide hexachlorphene (Fig 4-3).

Fig 4-2

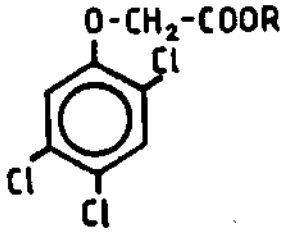
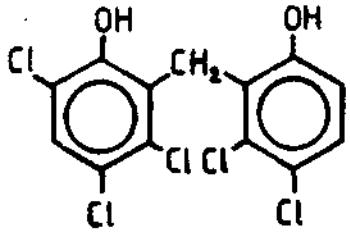


Fig 4-3



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; namely, by chlorination of the phenol using various catalysts and by the alkaline hydrolysis of the 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-trichlorophenol, see section 4.3.

PCDDs and PCDFs are also formed during the preparation of chlorinated diphenyls herbicides (Yamagishi et al, 1981) and hexachlorobenzene (Villaneuva et al, 1974). A series of PCDFs were formed during the production of PCBs, see section 4.3.

Production trains are often used for the production of chemicals whose manufacture necessitates the use of similar process equipment. In the manufacture of chemicals on a production train previously contaminated by PCDDs and PCDFs, both the products and waste generated can be contaminated. Thus, the manufacture of 2,4-D, which otherwise was not expected to be contaminated by

2,3,7,8-tetra-CDD did indeed contain this contaminant because the equipment used had employed in previous production of 2,4,5-T and the equipment remained contaminated (Federal Register, 1980).

It should be pointed out that the occurrence of TCDD in the environment can be mainly related to the synthesis of 2,4,5-trichlorophenol and the use of products prepared from this compound (Table 4.1) or incinerations reactions, while the occurrence of the other PCDDs and PCDFs can be related to the synthesis and use of a variety of other products (Table 4.2), some of which are quite common.

Table 4-1. List of commercial products that may be contaminated with 2,3,7,8-TCDD, 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-trichlorophenoxy acetic acid b)
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-trichlorophenoxy) - 2,2-dichloropropanoic acid
2,4,5-trichlorophenol	2,4,5-trichlorophenol
fenochlorphos	O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothionate
trichlonate	(O-ethyl O-2,4,5-trichlorophenyl ethyl phosphonotionate
hexachlorophene/isobac 20	2,2'-methylene-bis (3,4,6-trichlorophenol)

a) There are numerous trade names for this product

Table 4-2. List of commercial products that may be contaminated with other PCDOs 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-cyclor-hexadiene-1,4-dione.
2,4-D and esters and salts	(2,4-Dichlorophenoxy) acetic acid and esters and salts
2,4- OB and salts	2,4-Dichlorophenoxybutyric acid and and salts
Dicamba	3,6-Dichloro-2-methoxybenzoic acid
Dicamba, dimethylamine salt	3,6-Dichloro-2-methoxybenzoic acid, dimethylaminesalt
Dicapthon	Phosphorothioic acid o-(2-chloro-4-nitrophenyl) o,o-dimethyl ester
Dichlofenthion	Phosphorothioic acid o-2,4-dichloro-phenyl o,o-diakyl ester
Disul sodium (sesone)	2,4-Dichlorophenoxyethyl sulfate, sodium salt
2,4-DP	2- 2,4-Dichlorophenoxy prpionic acid
Nitrofen	2,4-Dichlorophenyl-p-nitrophenyl ether
Pentachlorophenol (PCP) and salts	Pentachlorophenol and salts

4.3 CONTAMINATION OF COMMERCIAL PRODUCTS

4.3.1 Chlorphenoxy acetic acid herbicides

Depending on the temperature control and purification efficiency, the levels of 2,3,7,8-tetra-COD may vary greatly in commercial products. For example the levels of 2,3,7,8-tetra-COD in drums of the herbicide Agent Orange placed in storage in the US and in the Pacific before 1970 have been 0.02 and 47 µg/g, see Table 4-3. More than 450 samples wer analyzed in this study, and the mean value

was 1.98 µg/g (Young et al, 1978). 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-tetra-CDD in individual 2,4,5-T preparations manufactural and used in the 1960's could be as high as 100 µg/g.

In analysis using high-resolution GC-MS and MS confirmation, Rappe et al (1978a) and Norström et al (1980) have reported that in other samples of Herbicide Orange, as well as in European and US 2,4,5-T formulations from the 1950s and 1960s, 2,3,7,8-tetra-COD was the dominating compound of this group (see Table 4-4). Only minor amounts of other PCDOs and PCDFs could be found, primarily lower chlorinated PCDDs in samples of Herbicide Orange. The European samples are possibly preparated by a low-temperature process, while the US sample is prepared by a high-temperature process (see section 4:2).

As a result of governmental regulations, efforts were made during the 1970's to control and minimize the formation of 2,3,7,8-tetra-COD during the 2,4,5-T production, and now all producers claim that their products contain less than 0.1 µg/g of 2,3,7,8-tetra-CDD.

Table 4-3. Concentration (μg/g) of 2,3,7,8-TCDD in samples of Herbicides Orange and Purple[a] (Young et al, 1978).

Source of Samples	Number of samples		Range of TCDD (μg)	Mean TCDD Concentration (μg)
	Orange	Purple		
Johnston Island Inventory, 1972[b]	200	(4)[c]	0.05-47	1.91
Johnston Island Inventory, 1974	10		0.07-5.3	1.68
NCBC, Gulfport Inventory, 1972[d]	42		0.05-13.3	1.77
NCBC, Gulfport Inventory, 1975	238		0.02-15	2.11
Eglin AFB Archived Sample		1[e]	-	45
Eglin AFB Inventory, 1972	2		-	0.04
The Weighted Mean Concentration of TCDD in Orange = 1.98 μg/g				

a Analyses for TCDD performed by Interpretive Analytical Services. Dow Chemical, U.S.A., Midland Michigan; Aerospace Research Laboratories, Wright-Patterson AFB, Ohio; and the Brehm Laboratory, Wright State University, Dayton Ohio.

b Surplus Herbicide Orange was shipped from South Vietnam to Johnston Island for storage in April 1972.

c Four of 200 samples may have been Herbicide Purple.

d The Naval Construction Battalion Center (NCBC) Gulfport, Mississippi served as a storage site for Surplus Herbicide Orange from 1969 to 1977.

e Herbicide Purple was extensively used in the evaluation of aerial spray equipment on Test Area C-52, Eglin Air Force Base Reservation, Florida, 1962-1964.

Table 4-4. Levels of 2,3,7,8-tetra-CDD in 2,4,5-T acid and 2,4,5-T ester formulations (Rappe et al, 1978a; Norström et al, 1980).

Sample	Origin	2,3,7,8-tetra-CDD (µg/g)
2,4,5-T acid	1952, Sweden	1.10
2,4,5-T ester	unknown, Sweden	0.50
2,4,5-T ester	unknown, Sweden	<0.05
2,4,5-T ester	1960, Sweden	0.40
2,4,5-T ester	1962, Finland	0.95
2,4,5-T ester	1966, Finland	0.10
2,4,5-T ester	1967, Finland	<0.05
2,4,5-T ester	1967, Finland	0.22
2,4,5-T ester	1967, Finland	0.18
2,4,5-T acid	1964, USA	4.8
2,4,5-T acid	1969, USA	6.0
Herbicide Orange	unknown, USA	0.12
Herbicide Orange	unknown, USA	1.1
Herbicide Orange	unknown, USA	5.1

4.3.2 Hexachlorophene

The bactericide hexachlorophene is prepared from 2,4,5-trichlorophenol, the key intermediate in the production of 2,4,5-T. Due to additional purification, the level of 2,3,7,8-tetra-CDD in this product is usually <0.03 mg/kg (Baughman and Newton, 1972). However, hexachlorophene also contains about 100 mg/kg of a hexachloroxanthene, the 1,2,4,6,8,9-substituted isomer (Göthe and Wachtmeister, 1972).

4.3.3 Chlorophenols

Chlorophenols are extensively used since the 1930s as insecticides, fungicides, mould inhibitors, antiseptics and disenfactants. The annual production volume is estimated to be in order of 150,000 tons. The most important use of 2,4,6-tri-, 2,3,4,6-tetra- and pentachlorophenol (or their salts) is for wood protection. 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, in paint, glues and outdoor textiles. 2,4-Di- and 2,4,5-trichlorophenol are used for the production of 2,4-D and 2,4,5-T herbicides (phenoxy acids), and hexachlorophene.

Chlorophenols are industrially produced either by direct chlorination of phenol or by hydrolysis of chlorobenzenes, the process being 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 biphenyl 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 PCOFs is of special significance because of their extraordinary toxicological properties (see Chapters 7-9).

Buser and Bosshardt (1976) reported on the results of a survey on the PCDD and PCDF contents in 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 (hexa-CDD < 1 yg) and a second series with much higher levels (hexa-CDD > 1 yg) of PCDDs and PCDFs. Samples of high PCDD contents had also high PCDF contents. For most samples, the contents of these contaminants were in the order: tetra- equal to penta- < hexa- < hepta- < octa-CDD/CDF. The ranges of the combined levels of PCDDs and PCDFs were 2-16 and 1-26 yg, respectively, for the first series of samples, and 120-500 and 85-570 yg, respectively, for the second series of samples. The levels of octa-CDD and octa-CDF were as high as 370 and 300 yg, respectively.

Some PCP-Na samples analyzed showed the unexpected presence of a tetra-CDD (0.05-0.25 yg), which was later identified by Buser and Rappe (1978) as the unusual 1,2,3,4-substituted isomer. Table 4-5 collects a number of relevant analyses of these products. The levels of PCDDs and PCDFs are much 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, a complete set of isomers has not been used in these investigations, and more research is necessary to identify all the isomers present for a risk evaluation of these products.

Table 4-5. Levels of PCDDs and PCDFs in commercial chlorophenols (g/g) (From Rappe et al, 1979)

	$2,4,6\text{-}Cl_3$	$2,3,4,6\text{-}Cl_4$	PCP	PCP
Tetra-CDDs	< 0.1	< 0.1	< 0.1	< 0.1
Penta-CDDs	< 0.1	< 0.1	< 0.1	< 0.1
Hexa-CDDs	< 1	< 1	< 1	2.5
Hepta-CDDs	< 1	10	0.5	175
Octa-COD	< 1	2	4.3	500
Tetra-CDFs	1.5	0.5	< 0.1	< 0.1
Penta-CDFs	17.5	10	< 0.1	< 0.1
Hexa-CDFs	36	70	0.03	< 0.3
Hepta-CDFs	4.8	70	0.5	19
Octa-CDF	< 1	10	1.1	25

4.3.4 Polychlorinated Biphenyls (PCBs)

Vos et al, (1970) were able to identify PCDFs (tetra- and penta-COFs) 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, that had previously been analyzed by Vos et al, (1970). They used packed columns and very few standard compounds and they reported that the most abundant PCDFs had the same retention time as 2,3,7,8-tetra-CDF and 2,3,4,7,8-penta-CDF. Using a complete set of PCDF standards and an isomer specific analytical method Rappe et al (1984) have reported on levels of 2,3,7,8-substituted PCDFs found in commercial PCB products, see Table 4-6.

4.3.5 Chlorodiphenyl ether herbicides

In 1981 Yamagishi et al, reported on the occurrence of PCDDs and PCDFs in the commercial diphenyl ether herbicides CNP, NIP and X-52. The values are collected in Table 4-7. Very few synthetic standards were used, but the major tetra-CDDs could be 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-tetra-CDD could be found in these samples (Table 4-6).

Table 4-6. PCDFs in commercial PCBs (ng/g).

PCB-typ	TRI- Tot	TETRA- 2378	Tot	12348 12378	PENTA- 23478	Tot	123479 123478	123678	HEXA- 123789	234678	Tot	HEPTA- Tot	Rec 2378 TCDD-%
Pyralene	700	53	630	10	T	35	ND	ND	ND	ND	ND	ND	79
A1254	63	19	1400	690	490	4000	2500	2100	190	130	10000	960	78
A1260	10	13	110	48	56	260	500	120	190	27	1500	1300	88
A30	500	35	573	14	28	160	50	59	ND	ND	220	T	79
A40	1300	180	2600	96	8	1700	79	68	ND	T	310	ND	79
A50	7400	3300	20000	760	1100	8000	700	360	18	98	3100	75	95
A60	770	840	6900	1100	990	8100	1600	330	170	330	6800	2000	95
T64	47	23	360	97	122	840	520	390	58	41	2600	220	72
Clophen C	710	54	1200	34	30	270	ND	T	ND	ND	T	ND	79
Blank	ND	NO	NO	ND	ND	ND	ND	ND	ND	ND	ND	ND	90
Blank	NO	ND	NO	NO	NO	ND	ND	ND	ND	ND	ND	ND	87

Table 4-7. Levels of PCDOs and PCOFs in commercial diphenyl ether herbicides (µg/g) (From Yamagishi et al, 1981).

	CNP	NIP	X-52
Tri-CDDs	ND	0.15	0.03
Tetra-CDDs	14.0	0.38	0.03
Penta-CDDs	37	0.05	0.01
Hexa-CDDs	0.8	ND	ND
Mono-CDFs	ND	0.34	0.48
Di-CDFs	0.35	0.12	0.21
Tri-CDFs	0.41	0.47	0.45
Tetra-CDFs	0.4	0.29	0.32
Penta-COFs	1.0	ND	0.08
Hexa-CDFs	0.2	ND	ND

ND = Not Detected

4.3.6 Hexachlorobenzene

Hexachlorobenzene is used for the control of wheat bunt and fungi. Villaneuva et al, (1974), analyzing three commercial hexachlorobenzene preparations, identified octa-CDD and hepta- and octa- CDFs, see Table 4-8. The levels and identity of the hepta-CDF was not given.

Table 4-8. Levels of octa-COD and octa-CDF (µg/g) in commercial hexachloro-benzenes (From Villaneuva et al, 1974).

Sample	Octa-CDD	Octa-CDF
A	0.05	0.35
B	< 0.2	2.33
C	211.9	58.3

4.4 Sources of environmental pollution

4.4.1 Industrial accidents

A series of accidents is described in the literature during the industrial production of 2,4,5-trichlorophenol. In most of these accidents the pollution of 2,3,7,8-TCDD has been to factories with circumscribed occupational exposure (section 9). However, on July 10, 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 appreciable quantities of 2,3,7,8-TCDD. This covered initially 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 2,3,7,8-tetra-CDD was released in this accident. About 80% of this amount was deposited in an area of 15 ha, at a distance of about 500 m from the plant. The levels of soil contamination in 3 zones are given in Table 4-9 CPocchiari, 1978).

Table 4-9, Distribution of TCDD contamination in the Seveso area on the basis of soil sample analyses.

Range (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

Zone A: high-level contamination, about 115 ha Zone B: low level contamination, about 255 ha. Surrounding area - about 1400 ha (Pocchiari 1978).

4.4.2 Improper disposal of industrial waste

In 1973, three horse arenas in Missouri were found to be contaminated by high levels of 2,3,7,8-tetra-CDD: the highest value was about 30 µg (Kimbrough et al, 1977). This contamination resulted from the application, in 1971, of waste oil to control dust at these locations. The dioxin had originated at a hexachlorophene-producing factor in Verona, Mo. Additional tri- and tetra-CDDs have also been found, but the major component was 1,2,4,6,8,9-hexachloroxanthene (Buser, 1978), 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-trichlorophenol or 2,4,5-T derivatives.

In 1982, numerous sites of potential 2,3,7,8-tetra-CDD contamination were discovered in eastern Missouri. The contamination originated from the same waste oil from the factory in Verona, Mo. The streets of the entire town of Times Beach, Mo., had been sprayed. More than 5200 soil samples from Times Beach have now been analyzed. Dioxin levels were higher than 1 ppb in 22% of the samples

and higher than 100 ppb in 2.2%: the highest value was 350 ppb (R.D. Kloepfer, US, EPA Region VII, Kansas City, Kan., personal communcation, cited in Rappe, 1984).

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

4.4.3 Massive use of chemicals

The Eglin Air Force Base in Nortwest Florida, USA has been used for the development and testing of aereal dissemination equipment for military defoliation operations. During the period 1962-1970 a 3 km^2 test area was sprayed with 73 ton of 2,4,5-T. Analyses of archived samples of the formulations indicated that approximately 2.8 kg of 2,3,7,8-tetra-CDD were applied as a contaminant of the herbicide. However, 2.6 kg of this TCDD were applied to a 37 ha test grid 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 on this site.

4.5 Other possible sources of PCODs and PCDFs in the environment

4.5.1 Thermal degradation of technical products

The formation of 2,3,7,8-tetra-CDD 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 yields approximately 1 g of 2,3,7,8-tetra-CDD per kg of 2,4,5-T salt, while no dioxin was identified from the samt 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-tetra-CDD was formed per kg of 2,4,5-T esters during combustion at 500-850°C. Two reports (Stehl and Lamparski, 1977; Andersson et al, 1978) have shown that 2,3,7,8-tetra-CDD could not be found on 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/kg 2,4,5-T burned, or less.

Rappe et al, (1978) have studied the burning of material impregnated with various salts of chlorophenols. Very carefully purified 2,4,6-tri- and penta-chlorophenate were studied in addition to a commercial formulation of 2,3,4,6-tetrachlorophenate. The analytical method uised in this study was not isomer specific, but the following conclusions could be drawn concerning the formation of PCDDs by thermal reactions:

- the expected dimerization products and products formed in the "Smiles re-arrangement" are the major PCDDs,
- no other thermal isomerization of the PCDDs formed can be observed;
- no formation of higher chlorinated PCDDs can be observed,
- octa-CDD and other higher chlorinated PCDDs yield lower chlorinated dioxins in a nonspecific dechlorination reaction. This is also a major reaction pathway,
- 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; Buser et al, 1978b) that all destruction of PCB contaminated waste using incinerators must be carefully controlled. In the temperature range 300-400°C, the yield of conversion seems to be in the part-per-million range (Morita et al, 1978), but Nagayama et al, (1981) have reported a dramatic increase of PCDFs at these temperatures in the presence of stainless steel and nickel.

Buser and Rappe (1979) studied the pyrolysis of 15 individual synthetic PCB isomers. This study 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 (> 1X) of PCDDs and PCDFs were formed. A complex mixture of isomers of PCDDs and PCDFs 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%. It has also been reported that pyrolysis of PVC yields higher chlorinated benzenes (Ahling et al, 1978).

A direct evidence for the conversion of PVC tc PCDDs and PCOFs has recently been reported by Marklund et al (1985). They found that Laboratory pyrolysis of PVC result in the formation of PCDDs and PCDFs, mainly hexa- and hepta-CDDs and Cl_4-Cl_7 PCDFs. In some cases the pattern of isomers seems to be very similar to those found in municipal and hazardous wastes incinerator, e.g. the hexa-CDFs.

The data discussed in this section is summarized in Table 4-10.

Table 4-10. Formation of PCDDs and PCDFs by thermal processes

Precursor	Conditions	Products
2,4,5-T salt	Pyrolysis	2,3,7,8-TCDD
2,4,5-T (vegetation)	Pyrolysis	No TCDD
	Burning	-"-
Cl-phenate	Burning	PCDDs* + PCDFs
PCDs	Pyrolysis	PCDFs**
PCBz	Pyrolysis	PCDFs + PCDDs ***
Cl-Diphenyl ethers	Pyrolysis	PCDFs + PCDDs
PVC	Pyrolysis	PCBz

* = PCDD formed by dimerization and a [illegible] decha[illegible]

** = other products: hexa- and penta-CBz

*** = products: PCBs, polychlorinated naphthalenes

4.5.2 Incineration of municipal waste

Emissions from incinerators, heating facilities and thermal power plants have for long time been the subject of much 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. In 1977 Lahaniatis et al, reported on the finding of chlorinated organic compounds in fly ash of a municipal incinerator. The compounds detected were chlorinated aliphatics, benzenes, PCBs and pesticides.

Also in 1977 Olie et al, reported on the occurrence of PCDDs and PCDFs in fly ash from three muinicipal incinerators in the Netherlands. Their results indicated the presence of up to 17 PCDD peaks, but not quantification or isomer identification was possible due to lack of synthetic standards. Buser and Bosshardt (1978) studied fly ash from a municipal incinerator and an industrial heating facility, both in Switzerland. In the former the levels of PCODs 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 have been published confirming the original findings of Olie et al (1977) and Buser and Bosshardt (1978). Up top now less data have been reported on the levels of PCDDs and PCDFs in other incineration products such as particulates and flue gas condensate, which are the true emissions.

A risk evaluation should be based on the levels found in the emissions of the 2,3,7,8-substituted PCDD and PCDF isomers found in isomer specific analyses using validated sampling and clean-up methods. However, in most studies non-validated methods are used and the results are given in terms of total levels of tetra-, penta-, hexa-, hepta- and octa-CDDs and CDFs.
The value of such studies is limited, especially in this case where the number of isomers is quite large. More than 30 PCDDs and 60 PCDFs have been found in fly ash samples. Other authors make the assumption of an equal distribution among the isomers. This seems to be an erroneous approach. Using the isomer specific analytical method described above, two samples from municipal waste incinerators were analyzed, (Figure 4-4). The Swedish incinerator (Eksj6) was operating by the fluidized bed technique, the Canadian by conventional Roster technique.

In both samples we could identify a series of PCDDs and also PCDFs. Of special interest is the observation that all tetra-CDDs found in these two samples co-elutes with the isomers present in a mixture of all the 22 tetra-CDDs (Rappe et al, 1983). A variation in the levels of the highly toxic 2,3,7,8-tetra-CDD was also observed (Figure 4-4). In the Swedish sample this isomer constitutes about 3% of the total level of tetra-CDDs while in the Canadian sample this isomer is less than 0.3%. This is a tenfold variation of this interesting isomer. Moreover, in these samples the ratio between the largest and the smallest peak is more than 100:1 (Rappe, 1984).

Studying the penta-CDDs (Figure 4-5) reveals that the interesting 1,2,3,7,8-penta-CDD is a middle constituent, while 2,3,7,8-substituted hexa-CDDs are minors (Figure 4-6).

Another interesting parameter to investigate for various incinerators is the PCDF/PCDD ratio (Table 4-11). At the Cl_4 and Cl_5 levels, the PCDFs are in general much more abundant than the PCDDs, while the situation is reversed at the Cl_7 and Cl_8 levels (Rappe et al, 1983b).

Table 4-11. PCDF/PCDD Ratios in Samples from Fluidized-Bed Municipal Incinerator.

	Condensates		Particulates	
	1	2	1	2
Cl_4	>500	>25	>100	>100
Cl_5	>30	>250	>60	>25
Cl_6	>300	>1000	>500	>200
Cl_7	1	2	1.2	0.8
Cl_8	0.36	0.68	0.60	0.83

Contrary to the PCDDs, where the 2,3,7,8-substituted isomers are normally minor (or middle 1,2,3,7,8-penta-CDD) constituents, the 2,3,7,8-substituted PCDFs are normally middle and/or major constituents in emissions from municipal incinerators (Figures 4-7, 4-8 and 4-9). The municipal incinerators can also be the sources of hazardous PCDFs.

Figure 4-7
4-8
4-9

In a report in 1978 it was proposed by scientists from Dow Chemical Company that PCDDs and especially 2,3,7,8-tetra-CDD are ubiquitous and formed as trace level byproducts of any normal combustion (Dow, 1978; Bumb et al, 1980). Consequently dioxins should have been present in the environment since the advent of fire ("The Trace Chemistries of Fire"). A recent survey of PCDD levels in particulate

from residental wood combustion units is quoted in support of the above (Nestrick and Lamparski, 1982). The survey showed PCDD levels in the pg/g-range. The major constitutent was octa-CDD, but tetra-CDDs (including the 2,3,7,8-isomer) was also found. The occurrence of penta-CDDs and all PCDFs is not discussed in these reports.

However, this hypothesis has been criticized (Anonymous, 1979, 1982). One of the main arguments is that coal-fired power plants are ruled out as a possible source of 2,3,7,8-tetra-CDD (Kimble and Gross, 1980; Junk and Richard, 1981). Another argument is that data are completely lacking in the Dow studies on levels of dioxin precursors (see Table 4-10) in the material being burned, including the air in the flames.

4.5.3 Incineration of hazardous waste

Analyses from a test burn of pentachlorophenol waste has been reported by Rappe et al (1983). PCP is a well known precursor to octa-CDD, see section 2. Samples of baghouse ash and bottom ash were analyzed. In the baghouse ash the total level of octa-CDD was only 0.2 µg/g. The major constituents were lower-chlorinated PCDDs like hepta-, hexa-, penta- and tetra-CDDs. The isomeric distribution was reported to be very similar to a "normal" fly ash, in both cases 2,3,7,8-tetra-CDD is 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 Cl_4 and Cl_5 equal amounts of PCDDs and PCDFs were reported. The PCDFs can not be formed in a normal dimerization reaction. No PCDFs were found in the bottom ash. The authors suggest a difference in the formation pathway between PCDDs and PCDFs.

4.5.4 Wire reclamation

Hryhorczuk et al (1981) studied a wire reclamation incinerator in the US. Using a non-isomer specific analytical method, they analyzed for total levels of tetra-CDDs and tetra-CDFs. Two samples were analyzed, one from the furnace and one from the stack. The furnace sample contained 58 ng/kg totally of TCDDs and 730 ng/kg totally of TCDFs, the stack sample contained A10 ng/kg totally of TCDDs and 11 600 ng/kg totally of TCDFs.

4.5.5 Fires in PCB filled electrical equipment

In february 1981 a fire in the State Office Building in Binghamton, NY, USA caused a transformer to rupture, releasing soot in the whole building. The dielectric fluic in the transformer consisted of a mixture of PCB (65%) and chlorinated benzenes (35X).

The soot was found to be highly contaminated by PCDFs, the total level of PCDFs was more than 2000 μg/g. The results of the analysis are compiled in Table 4-12. The most toxic isomers (2,3,7,8-tetra-CDF, 1,2,3,7,8- and 2,3,4,7,8-penta-CDF and 1,2,3,4,7,8- and 1,2,3,6,7,8-hexa-CDF) were found to be the major constituent within each group of congeners. In addition a series of PCODs could also be identified including a large amount of the highly toxic 2,3,7,8-tetra-CDD and 1,2,3,7,8-penta-CDD. (Rappe et al, 1983b; Buser and Rappe, 1983). It is assumed that the chlorinated benzenes were the dioxin precursors.

During 1981-1985 a series of capacitor accidents have occurred in Scandinavia. The accidents were of two kinds: capacitor explosions[1] or capacitor fires where mineral oil filled capacitors were also involved[2]. In all cases the accident sites were contaminated by PCOFs (see Table 4-12).

4.5.6 Photochemical formation

The photochemical dimerization of chlorophenols to PCDDs has been studied by Crosby and Wong (1976). The only PCDD formed in this study was the octa-CDD. Other PCDDs can be formed by photochemical cyclization of chlorinated o-phenoxy-phenols, also called predioxins (Nilsson et al, 1974). These predicxins 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 predioxin.

Åkermark (1978) studied the formation of 2,3,7,8-tetra-CDD form the appropriate predioxin. They could identify the product, bJt they claim the reaction is very inefficient.

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[1] Stockholm, Hallstahammar and railway loccomotive.
[2] Skövde, Surahammar.

Another photochemical process of potential environmental importance is dechlorination of the higher chlorinated PCODs and PCDFs, octa-CDD and octa-CDF. The products formed in solution photolysis of octa-CDD have now b een identified (Buser and Rappe, 1978). By comparison with authentic standards it was found that the main tetrachloro isomer was the 1,4,6,9-tetra-CDD; the major penta-chloro compound is expected to be the 1,2,4,6,9-isomer. 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-isomer, respectively. The reaction scheme deduced from this data shows that the chlorine atoms are removed preferably from the lateral positions on the carbon rings. Consequently the most toxic PCDD isomers such as 2,3,7,8-tetra-CDD are not likely to be formed from the solution photolysis of the higher PCDDs.

Crosby and Moilanen (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.

5. ENVIRONMENTAL TRANSPORT AND TRANSFORMATIONS

5.1 Environmental transport

5.1.1 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 modeling (SAI, 1980). In the case of the accidental release of a toxic cloud containing 2,3,7,8-TCDD at Sevèso, Italy, Cavallaro et al (1982) determined the transport pattern and the ground deposition. They determined that the deposition of 2,3,7-8-TCDD from air to soil should follow an exponential decay pattern in the Gaussian-distribution along the cross-section of the downwind direction. Thibodeaux (1983) has studied the air transport of 2,3,7,8-tetra-CDD at a herbicide production facility in Jacksonville, Arkansas, USA.

5.1.2 Water

The solubility of 2,3,7,8-TCDD in water is 0.2 ng/ml and no data is available for the other PCDDs and PCDFs. However, data from microbiological experiments indicate that 2,3,7,8-TCDD is highly sorbed to sediments and biota. Mutsumura et al (1983) suggest that more than 90% of the 2,3,7,8-tetra-CDD in an aquatic medium may be present in the adsorbed state. Rappe et al (1984) 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-tetra-CDD transported by a creek in the contaminated herbicide factory in Jacksonville, Arkansas. The value was 0.89 g/year as an average rate and maximum of 2.1 g/year.

5.1.3 Soil and sediments

The mobility of TCDD and of a dichlorodioxin in soils has been studied (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 in north-west Florida which had been heavily sprayed with the herbicede "Agent Orange" between 1962 and 1964 (Young et al 1975). This herbicede mixture was contaminated with TCDD (section 4.2). A 19.3 acre test grid received a total of 40 metric tons of 2,4,5-T between 1962 and 1964. When 6-inch core soil 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 hebicide application. Similar TCDD concentrations were obtained from areas that had been sprayed between 1962 and 1969 (Bartleson et al 1975).

In another study Young (1983) studied the concentration of 2,3,7,8-TCDD in a soil profile. The samples were collected in 1974 and the data suggested that most of the 2,3,7,8-TCDD would be found in the top 15 cm of the soil profile, Table 5-1.

Table 5-1. Concentration of 2,3,7,8 in a soil profile (Young, 1983)

Depth (cm)	TCDD ng/kg
0 - 2.5	150
2.5 - 5.0	160
5.0 - 10	700
10 - 15	44
15 - 90	ND[b]

[a]Grid I received 1,069 kg/ha of 2,4,5-T Herbicide during 1962-1964. The soil samples were collected and analyzed in 1974.
[b]None detected, minimum detection limit 10 ng/kg.

The probable media and modes of transport of polychlorinated dioxins 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 sorbed PCDDs and PCDFs has been considered to be a much more important transport meachism than leaching and volatilization because of the low water solubility and volatility of these compounds (Josephson, 1983). However, the monitoring of Seveso soil 1 year after the accident showed that the highest 2,3,7,8-TCDD 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-TCDO 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 polychlorinated dioxins from soils under certain special situations, namely heavy rainfall and with sandy soils. Studies by Young (1983) indicate that the half-life for 2,3,7,8-tetra-CDD in soil is 10-12 years.

Thibodeaux (1983) has studied the vaporization of 2,3,7,8-tetra-CDD from a herbicide plant in Jacksonville, Arkansas. The vaporization can take place from soil surface, from landfill cells and from surface of a pond, Table 5-2.

Table 5-2. Surface source areas and emission rate summary (Thibodeaux, 1983)

Source	Area (m^2)	Emission rate (g/yr)
Blow-out area, volatilization	753	120.-1,200
Blow-out Area, entrainment	753	28.-37.
Rocky Branch Creek, dissolved		0.89-2.1
Reasor-Hill dump	1,129	0.1-1.0
Rocky Branch Creek, sediment		0.094-0.22
Cooling water pond	15,050	0.015-0.016
Total		150.-1,240

It was found that vaporization from the soil surface in the highly contaminated blow out area is the major contributing source.

Freeman and Schroy (1984) have developed a model to describe the vaporization and diffusion of low volatility organic chemicals like PCDDs and PCDFs through a column of soil. This model has been used to make predictions on the transport of 2,3,7,8-tetra-CDD at a site in Times Beach, MO, USA. The model predicts that the 1983 levels in this soil are only 10% of the original loading. The model also predicts that 57% of the initial amount of 2,3,7,8-tetra-CDD was vaporized through the soil column to the surface in the first year after the spraying and most of the vapor transport occurs during the summer months.

5.2 Environmental transformation

5.2.1 Abiotic transformation

TCDD, like other PCDDs and PCDFs, is chemically quite stable, and is not likely to be degraded at a significant rate by hydrolytic reactions under environmental conditions.

Under environmental conditions TCDD seems also to be rather stable to photochemical degradation. The half-time of TCDD of about half a year found by Young et al (1976) for soil is in agreement with this statement. Moreover, Crosby et al (1971) found the photochemical dechlorination of TCDD to be extremely slow on the soil surface.

However, three reports on rapid photochemical degradation of TCDD 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 experiments 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 and Wong 1977). In these two experiments a "hydrogen donor", such as methanol, or 2,4,5-T ester, highly enhanced the photochemical dechlorination (Åkermark 1978), so that these two experiments do not truly reflect environmental conditions, where the 2,4,5-T ester is rapidly hydrolyzed on the surface of the leaves. It can be mentioned here that at Seveso the TCDD was released together with salts of 2,4,5-trichlorophenol, ethylene glycol and inorganic constituents (Rappe 1978). 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-hexadecylpuridinium chloride was also reported to enhance photodecomposition (Botre et al 1978).

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

In order to explain the longer half-life of 2,3,7,8-TCDD in a model laboratory ecosystem than in an outdoor pond, Matsumura et al (1983) speculated photolysis as the most likely cause. In the outdoor environment where the intensity of sunlight was higher comparaed to the laboratory experiments, algae-mediated photosensitization of 2,3,7,8-TCDD may cause 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-TCDD in methanol occurred in 24 hours under UV irradiation, >80% octa-CDD 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 pattern also plays a critical part. In higher polychlorinated dioxins, there appears to be preferential loss of chlorine from the 2, 3, 7 and 8 positions (Buser and Rappe, 1978). Thus, polychlorinated

dioxin compounds with chlorine substitutions in positions 2, 3, 7 and 8 are likely to photodegrade faster than compounds not having these positions substituted. For example, the photolysis half-life of 1,2,3,7,8-PeCDD has been estimated to be 7.8 hours in n-hexadecance solution under sunlamp irradiation (Nestrick et al, 1980). Similarly, the photolytic half-lives of 1,2,3,7,8-PeCDD, 1,2,3,6,7,9- and 1,2,4,6,7,9-HCDD in hexane solutions under sunlight irradiation have been determined to be 5.4, 17 and 47 hours, respectively (Dobbs and Grant, 1979). Nestrick et al (1980) reported a half-life value of 6.8 hours for 1,2,3,6,7,8-HCDD in n-hexadecane under sunlamp irradiation. The primary intermediater of photodegradation of higher polychlorinated dioxins are probably lower chlorinated dioxins (Buser, 1976), but the pathways of degradation are not known with certainty (National Research Council of Canada, 1981).

From these discussions of the photolysis of polychlorinated dioxins 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 unlike in solution, a predominant amount of polychlorinated dioxins in surface water may remain sorbed or suspended on particles and sediments. Moreover, since the penetration of UV light into natural water may be very limited, photolytic degradation of polychlorinated dioxins is not likely to be of environmental importance.

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

5.2.2 Biotransformation and biodegradation

2,3,7,8-TCDD exhibits relatively strong resistance to biodegradation. Only 5 of about 100 microbioal strains that have the ability to degrade persistent pesticides show slight ability to degrade 2,3,7,8-TCDD (Matsumura and Benezet, 1973). Ward and Matsumura (1977) studied the biodegradation of ^{14}C-labeled 2,3,7,8-TCDD in Wisconsin lake waters and sediments. The observed half-life of 2,3,7,8-TCDD in sediment-containing lake waters was found to be 550-590 days. In lake water alone, about 70% of the 2,3,7,8-TCDD remained after 589 days. Using an outdoor pond as a model aquatic ecosystem and dosing it with ^{14}C-labeled 2,3,7,8-TCDD, Matsumura et al (1983) estimated the apparent half-life of 2,3,7,8-TCDD 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-TCDD in aquatic systems from this experiment.

In 1982 Philippi et al reported on the occurrence of a polar metabolite of 2,3,7,8-tetra-CDD, which was found in several microbiological cultures after long term incubation. The authours report chromatographic and MS data which support the conclusion that the metabolite is 1-hydroxy-2,3,7,8-tetra-CDD, although the sythetic standard compound was not available.

Tulp and Hutzinger (1978) reported that in rats, dibenzo-p-dioxin, 1-chlorodibenzo-p-dioxin, 2-chlorodibenzo-p-dioxin, 2,3-dichlorodibenzo-p-dioxin, 2,7-dichlorodibenzo-p-dioxin, 1,2,4-trichlorodibenzo-p-dioxin and 1,2,3,4-tetrachlorodibenzo-p-dioxin are metabolized to mono- and dihydroxy derivatives, whilst in case of dibenzo-p-dioxin and both the two monochloro isomers, also sulphur containing metabolites are excreted. Primary hydroxylation exclusively takes place at the 2-, 3-, 7- or 8-position in the molecule. In none of the experiments metabolites resulting from fission of the C-0 bonds (ortho, ortho'-dihydroxychlorodiphenyl ethers, chlorocatechols) of hydroxylated derivatives thereof, were detected. No metabolites were found from octachlorodibenzo-p-dioxin.

5.3 Bioaccumulation

Bioaccumulation of ^{14}C-TCDD was investigated in two 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 more than 9000 times. Under similar conditions, the level in shrimps (Artemia salina) was 1570 times higher than that found in water (Matsumura and Benezet 1973). In the second study (Isensee and Jones 1975, and Isensee 1978) ^{14}C-TCDD was absorbed, at a broad range of levels, to soil and placed at the bottom of an aquarium. Five species of organisms (Table 5-3) 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 0.94 or higher). As with most pesticides, the lower food-chain organisms (algae and daphnids) contained greater concentrations than higher food-chain organisms (snails and Gambusia).

5.4 Levels in biota

5.4.1 Vegetation

When ^{14}C labelled TCDD 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 and Jones 1971). A maximum of 0.15% of the TCDO present in the soil was translocated to the aerial portion of the oats and the soya beans, but neither the grain nor the soya beans harvested at maturity showed any level of ^{14}C labelled TCDD. When TCDD was applied to the centre 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, gave values up to 50 mg/kg TCDD (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 and 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, nor in corn cobs and kernels grown near the factory. The detection limit for the analysis was reported as 1 pg/g. At the same time about 100 pg/g of dioxin was detected in the peels of fruits. This strongly suggests that the contamination is due to dust and not from plant uptake. The dioxin level in the soil was found to be in the order of 10 ng/g, about 1000 $\mu g/m^2$ (Wipf et al, 1982).

Very few analyses of sprayed vegetation has been reported. A rough estimate of 2,3,7,8-tetra-CDD contamination of 20-1000 pg/g 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-tetra-CDD in the spray formulation used, ever- higher values could be obtained if Agent Orange is used. Sundström et al (1979) reported on data in agreement with this estimation. However, the analytical technique used in this study was not isomer specific. Vegetation was sprayed with 2,4,5-T ester contaminated by only 0.06 $\mu g/g$ of 2,3,7,8-tetra-CDD. A sample of leaves collected 42-45 days after the spraying was found to have 170 pg/g of TCDD, somewhat lower than the expected value (600 pg/g) indicating a slow photochemical breakdown.

5.4.2 Aquatic organisms

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

In two creeks associated with the test area in north-west Florida (section 5.1) which had been heavily sprayed with "Agent Orange" between 1962 and 1964, 10 years later the silt contained 10 and 35 ng/kg TCDD where eroded soil entered the water. Concentrations of 12 ng/kg TCDD were found in 2 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/kg TCDD in skin and muscle, 18 ng/kg in the gonards and 85 ng/kg in the gut (Young et al, 1976).

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 to 400 ng/kg of 2,3,7,8-tetra-CDD in Bayou Meto/Arkansas River, a waterway associated with the production of 2,4,5-T (Thibodeux, 1983), see Table 5-4.

Levels ranging from 0.004-0.695 γg/kg were found for the edible portion of channel catfish, carp, yellow perch, smallmouth bass, sucker from Saginaw Bay. The highest concentrations were detected in bottom-feeding catfish and carp, and the lowest concentrations were detected in bass, perch and suckers, see Table 5-5 (Harless and Lewis, 1982).

Table 5-5. Analytical results for 2,3,7,8-TCDD residues in fish (Harless and Lewis, 1982)

Species	No. samples	No. positive samples	Sample preparation efficiency[6]	TCDD detected (ng/kg)[7] low	high	mean	TCDD minimum limit of detection
Channel Catfish	8	8[1]	87	28	695	157	13
Carp	14	10[2]	78	20	153	55	7
Yellow Perch	6	33	78	10	20	13	5
Small Mouth Bass	2	2[4]	100	7	8	8	6
Sucker	4	35	92	4	21	10	4
Lake Trout	2	0	100	0	0	0	5

1 Tittabawassee—4, Saginaw—2, Saginaw mouth—1, Grand—1
2 Tittabawassee—5, Saginaw—2, Saginaw mouth—1, Grand—2
3 Tittabawassee—2, Saginaw mouth—1 (composite of 2 fish for each analysis)
4 Grand—2
5 Tittabawassee—2, Saginaw Bay—1
6 Mean % recovery for 2.5 to 10 ng $^{37}Cl_4$-TCDD added to 5 or 10 g samples prior to sample preparation.
7 Corrected for lossess in sample preparation efficiency.

Rappe et al (1981) identified a series of tetra to octa-CDFs in fat samples of a snapping turtle from Hudson River and gray seal from 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-hexa-CDF).

Norstrom et al (1982) have analyzed pooled samples of herring gull eggs collected in 1982 from various parts of the Great Lakes. In all samples 2,3,7,8-tetra-CDD was found in levels ranging from 9 to 90 pg/g. 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 identify measurable levels of tetra-CDDs and other PCDDs in fish samples from Lake Superior (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 contrary a series of PCDFs could be identified in the Lake Superior fish samples, indicating a more widespread background levels for the PCDFs than for the PCDDs. Stalling et al (1983) also reported on the analysis of fish samples from Lakes Michigan, Huron and Ontario.

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 Tittabawasee River. The residue pattern found in the fish and locally high levels suggest a strong influence by point source discharges. The recent data by O'Keefe et al (1983) is also in agreement with this statement.

Ryan et al (1983) analyzed for 2,3,7,8-tetra-CDD in Great Lakes a series of commercial and sport fish from Canada and also from the Pacific coast, see Table 5-6. The highest levels were found in Lake Huron and Lake Ontario. In a preliminary study they also report on 3-200 ng/kg of 2,3,7,8-tetra-CDFs and other unidentified tetra-CDFs.

Table 5-6. 2,3,7,8-Tetra-CDD and PCB levels in sport fish (1980) and smelt (1979) (Ryan et al, 1983)

Species	Origin	TCDO ng/kg	PCB Yg/g
Lake Trout[a]	Lake Ontario	58	7.28
	Lake Huron	37	5.03
Rainbow Trout[a]	Lake Ontario	33	1.77
Coho Salmon	Lake Ontario	28[b]	7.39
	Pacific Coast	ND[c] (A)	0.03
Smelt	Lake Ontario	11	
		16	
		11	
	Lake Erie	ND[c] (2)	

[a] Whole fish.
[b] Also contained 36 ng/kg of hexa-CDO (three isomers) and 93 ng/kg of octa-CDD.
[c] NO = not detected at backeted detection limit.

Rappe et al (1985) have identified a series of Cl_4-Cl_6 PCDFs and PCDDs in samples from the Baltic Sea. The major components were the 2,3,7,8-substituted isomers, see Table 5-7.

5.4.3 Terrestrial animals

In the heavily sprayed testarea 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). Liver from the beach mice contained from 540-1300 ng/kg TCDD and the pelt 130-140 ng/kg. The visceral mass of race runners (Cnemidophorus sexlineatus) which were caught in that area contained 360 ng/kg TCDD and the trunk of the reptiles contained 370 ng/kg.

At the time of the accident in Seveso more than 81 000 animals were inhabitating in the contaminated zones. The main part were rabbits (25 000) and poultry and other small animals (55 500), but also 349 cattle, 233 pigs 49 horses, 21 sheeps and 49 goats were in the zones. Many of these animals died other were killed. A large number of these animals were analyzed for 2,3,7,8-TCDD by a method with detection level at 250 ng/kg. The results are collected in Table 5-8 and 5-9.

Table 5-8. TCDD analyses on liver of farm animals from contaminated zones and surrounding areas (1976-1979)[a] (Pocchiari et al, 1983)

Animal	Number of samples	TCDD-contaning samples	TCDD maxmium 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] Data by Veterinary Service of Lombardy Region et al (1980).

[b] Figures include rabbits kept in the special test plots on contaminated ground for experimental purposes.

Table 5-9. TCDD analyses on wildlife animals from contaminated zones and sorrounding areas (1976-1979) (Pocchiari et al 1983)

Animal	Tested organs and number of samples	TCOO-containg samples	Maximum level of TCOO ng/g
Hares	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

Harless et al (1983) report on a study where 2,4,5-T containing less than 0.1 mg/kg of 2,3,7,8-TCDD was applied at a rate of 3.4 kg per hectare to approximately 3 hectare of the enclosed plot (4.5 hectare). 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. Oeer were sacrificed prior to and at specific intervals during the course of the 30 day study. The analytical results are summarized in Samples from a horse grazing close to a wire reclamation incinerator were analyzed and found to contain unspecified Tetra-COFs (165 ng/kg in the fat, 57 ng/kg in the liver) and unspecified tetra-CDOs (45 ng/kg in the fat and lass than 6 ng/kg in the liver (Hryhorczuk et al, 1981).

5.4.4 Human data

Occupational exposure to 2,3,7,8-tetra-CDD can occur during the production of 2,4,5-trihclorophenol and the subsequent production and use of 2,4,5-T acid and esters. The 2,4,5-T was first produced commercially in 1944 in United States and the use of 2,4,5-T as herbicide increased in the 1940's and 1950's. However, the problem of dioxin contamination in 2,4,5-T was not recognized until 1957 (Kimmig and Schulz, 1957).

The most heavy exposure after a normal production is during a purification step as the residues are by far more contaminated than the purified products. Only limited information is available on the levels of dioxin contamination of

products prepared prior to the 1970's, 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 exposure during this period.

The dioxin exposure of soldiers and the general population during the military use of phenoxy herbicides during the Vietnam war has been the subject of much concern both among the Veterans in the United States,Australia and New Zealand as for the people in Vietnam. Young et al (1983) discuss a study where very low levels of tetra-CDD, believed to be the 2,3,7,8-isomer, was detected in adipose tissue from many of the Vietnam Veterans, although the levels are not considered to correlate well with known exposure data or with health status. No experimental conditions are given in this report.

Facchetti et al (1980) studied tissue samples from a 55 year old woman, who lived in the most contaminated zone (A) in Seveso, Italy. The accident occurred July 10 adn the woman remained in her home until July 26. The death occurred 7 months after the accident. The results are collected in Table 5-11.

Analysis of blood plasma can be 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 (1983c) have investigated such exposure through the analyses of blood plasma of exposed workers and unexposed controls. Good correlation was found between the plasma levels and

1) the nature of exposure
2) the duration of exposure
3) the isomers present in the commercial formulation

In 1968 more than 1500 persons in southwest Japan were intoxicated by consumption of a commercial rice oil accidentally contaminated by PCBs, PCDFs and polychlorinated quaterphenyls (Masudaet al 1982, Miyataet al 1978). In 1979 a similar episode occurred in central Taiwan, the number of persons involved here approaching 2000 (Chen et al 1980). Both these accidents have been referred to as Yusho episodes, hut recently Taiwanese episode is renamed Yu-cheng. The Japanese rice oil contained more than 40 PCOF isomers, tri- to hexa-CDFs. Analyses of liver samples taken from the Japanese patients about 18 months after the exposure showed s dramatic decrease in the number of PCDF isomers. Apparently most of the PCDF isomers have been 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 in the liver samples revealed an interesting relationship. Most of the isomers retained had all lateral positions (2-, 3-, 7- and 8-) substituted for chlorine, these isomers have the highest toxicity.

The rate of excretion of these toxic dibenzofurans isomers is very slow, as studied by the Japanese and Taiwanese Yusho patients. A 15-20% reduction in the blood levels was found in one year (Rappe, 1984), and PCDFs could still be found 11 years after the exposure in Japan (Rappe et al, 1983c). This indicates a dramatic difference in the rate of excretion between nan and rodents, where the half life of 2,3,7,8-tetra-CDD is reported to be only a few weeks.

Ryan and Williams (1983) recently reported on the levels of 2,3,7,8-tetra-CDD in 23 autopsy samples of adipose tissue from the Great Lakes area in Canada. The analytical method used was capable of separating the 2,3,7,8-isomer with a possible 1-3 other co-eluting isomers. The 2,3,7,8-tetra-CDD was found in 22 of the 23 samples and values ranged from 4.1 to 130 ng/kg. Excluding the outlaying high sample the average value was 10.7 ± 5.4 ng/kg.

A study including the analysis of 31 samples of human adipose tissue has recently been reported (Rappe, 1985). In all samples a series of 2,3,7,8-substituted PCDDs and PCDFs were found in levels ranging from a few pg/g up to 400 pg/g. The mean values are given in Table 5-12.

The same 2,3,7,8-substituted isomers at approximately the same levels (counted on fat weight basis) have also been found in a few samples of mather's milk from N Sweden and Germany, see Table 5-12 (Rappe, 1985).

6. ENVIRONMENTAL LEVELS AND EXPOSURES

6.1 Air

Owing to analytical problems, no data is available on the levels of 2,3,7,8-TCDD and other PCDDs and PCDFs in normal urban air.

Airborne dust was monitored in 1977 in the Seveso area to evaluate the possibility that 2,3,7,8-contaminated particles could be outside the contaminated areas. High volume sampling technique was used. When pooled particulate samples were analyzed levels of 0.17 pg/m^3 - 0.50 pg/m^3 are reported (Wipf et al, 1982).

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

Rappe et al (1984) have analyzed indoor air samples from fires and explosions in PCB filled electrical equipment, see Table 6-1.

Table 6-1. Analyses of PCDFs in air samples (pg/m^3) (Rappe et al 1984)

Sample	Cl_4	2378-	Cl_5	Cl_6	Cl_7	Cl_8
Surahammar During cleaning	<20	< 2	<10	<10	<10	<10
Surahammar After cleaning	<10	< 2.5	<10	<10	<10	<10
Railway locomotive During cleaning	500	50	50	30	20	20

6.2 Water and leachate

Shadoff et al (1977) failed to identify 2,3,7,8-tetra-CDD 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, ArcKansas, USA, Thibodeaux could not detect any 2,3,7,8-tetra-CDD in the creek water (no detection levels given).

In Seveso, since August, 1976 a number of tests have been periodically conducted on running water streams in 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 within the first few kilometers downstream from their confluence were obtained. Negative results were obtained farther downstream. 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; 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/l (parts per quadrillion) (Pocchiori, 1983).

In 1983 leachates from the dumpsite Georgswerder in Hamburg was reported to contain 2,3,7,8-tetra-CDD at a level of about 100 pg/g, the analytical method was not specified (Freie und Hansestadt, Hamburg, 1984).

6.3 Soil and sediment

In 1973 it was found that three horse arenas in Missouri were contaminated by high levels of 2,3,7,8-tetra-CDD, the highest value was about 30 µg (Kimbrough et al, 1977). This contamination resulted from the application in 1971 of waste oil to control dusting at these locations. The dioxin had originated at a hexachlorophene producing factory in Verona, MO. Additional tri- and tetra-CDDs have also been found, but the major components was 1,2,4,6,8,9-hexachlor-oxanthene (Buser, 1978) a compound which apparently can severe as a marker for this type of contamination. The xanthene is a normal by-product in the hexa-chlorophene production and has never been associated with the production of 2,4,5-trichlorophenol or 2,4,5-T derivatives.

In 1982, numerous sites of potential 2,3,7,8-tetra-CDD contamination were discovered in Eastern Missouri. The contamination originated from the same waste oil from the factory in Verona, MO. In the town of Times Beach, MO, the streets of the entire town had been sprayed. Now, more than 52 00 soil samples have been analyzed from Times Beach. The dioxin levels were higher than 1 ppb in 22% of the samples and higher than 100 ppb in 2.2%, the highest value was 350 ppb (Personal communication R.D. Kloepfer, US EPA Region VII, Kansas City, Kansas, cited by Rappe (1984).

Another Location of great concern is Love Canal, Niagara Falls, NY. Here Smith et al (1983) also found high levels of 2,3,7,8-tetra-CDD in storm sewer sediments taken from around the Love Canal waste disposal site, the highest value was 312 ppb.

Analytical results of the 1976/77 survey for the Zones B and R in Seveso are dicussed 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 elaboraion of the relevant data, indicate 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 of Zone R and five sites outside Zone R were analyzed using a high resolution GS-MS system to establish whether isomers of 2,3,7,8-TCDD were present. A significant percentage of TCDD decrease can be accounted for by two TCDD isomers (1,3,6,8-TCDD and 1,3,7,9-TCDD) present in the majority of the samples tested. These two isomers are not related to the chemical accident at the ICMESA factory.

Wipf et al (1983) report on a series of PCDDs in the soil from Zone R, Table 6-2. They suggest that a municipal incinerator and burning wood shavings treated with chlorinated phenols can be the source to the other PCDDs.

Table 6-2. PCDD contamination in soil from Zone R (1981) (Values in pg/g) (H.K. Wipf and J. Schmid).

Sample	2,3,7,8[a]	TCDD[b]	PCDD	HxCDD	HpCDD	OCDD	Total
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] 2,3,7,8-TCDD, probably related to accident

[b] Isomers other than 2,3,7,8-TCDD, not related to accident

In a recent study Czuczwa and Hites (1984) have found a series of PCDDs and PCDFs in sediment samples from several locations in Saginaw River and Bay and southern Lake Huron. The levels ranged from 100 ppb in urban areas to 100 pg/g at remote sites. Although no isomers were identified, the analytical profiles in the sediments were reported to follow closely to 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 levels at a depth corresponding to approximately 1940 and remaining high up to now. There is no good correlation between the trend in these levels and the trend for coal burning in the United States, which can be predicted from the hypothesis of "Trace Chemistries of Fire". However, the levels in the sediments agree well with the production of chlorinated aromatic compounds.

6.4 Food

The US Environmental Protection Agency (EPA) initiated a TCDD 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 Chemicals Co. (USA), Wright State University and Harvard University. All the laboratories used mass spectroscopic techniques for quantification. Two different extraction techniques were used. Control samples were taken from cattle that had grazed in non-treated areas, and control samples with known amounts of TCDD were also analyzed by the 3 laboratories. All the controls were prepared by the EPA (EPA 1976; Firestone 1978).

Good agreement was found between the levels of TCDD added to the control beef fat samples and the levels found, down to the added TCOD levels of 10 ng/kg. The average reported TCDD level was 10 ng/kg; the level actually added by the EPA was 9 ng/kg. Of a total of 3A analyses of controls to which no TCDD was added, there was only one case of a positive report on 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 range of average reported levels 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 one pound of 2,4,5-T per acre, all 19 positive samples (48%) belong to this group, and the average reported TCDD level would be 9 ng/kg. The results gave an indication of a consistent trend relating the average reported TCDD level in beef fat to the intensity of the 2,4,5-T application on treated rangeland (O'Keefe et al 1977). Some of these data can also be found in an EPA memorandum (EPA 1976) and in Firestone (1978) and McKinney (1978).

It is to be noted that none of the 3 collaborating laboratories used the most selective and sensitive analytical method now known (capillary glass column gas chromatography - mass fragmentography). It is recommended that these beef fat samples be re-analysed using this latter method.

Kocher et al (1978) analyzed specimens of fat taken from steers which had grazed on rangeland previously treated with 2,4,5-T herbicides were analyzed for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The limit of detection of TCDD (2.5 times peak to peak noise) was found to be in the 30-60 picogram range (3-6 pg/g in beef fat using 10 gram samples). None of the sixteen samples comprising two of the three studies showed any response for TCDD. In the third

study, in which the animals were confined to a fenced pasture sprayed in its entirety with a 2,4,5-T herbicides, samples from three of the seven animals gave a positive response at the extremely low level of 3 to 4 pg/g TCDD, which is at the detection limit, the highest reported value (without inferering components) was 13 pg/g. The level of 2,3,7,8-tetra-CDD in the 2,4,5-T used was however unknown.

Using the direct probe-high resolution mass spectroscopy technique, O'Keefe has found low levels of TCDD (in the range of ng/kg (pg/g)) in a few samples of mother's milk from areas where 2,4,5-T had been used (O'Keefe - personal communication, quoted by Allen et al 1977). This report has still to be confirmed. On the other hand, Shadoff et al (1977) and Mahle et al (1977) failed to identify and quantify TCDD in mother's milk and bovine milk from farms where 2,4,5-T had been used (analytical technique: packed column -high/low resolution MS). Lamparski et al(1978) have analyzed samples of quarantiened milk from Michigan, USA. The found no hepta-CDD and octa-CDD at a detection level of 25 pg/g and 50 pg/g respectively.

Tissues and milk from cattle possibly intoxicated by licking pentachlorophenol treated timber has been analysed for higher chlorinated PCDDs. Hexa-CDDs, hepta-CDOs and octa-CDD were identified in the ng/kg (pg/g) range (Moore 1977).

Ryan and Pilon (1982) have studied the levels of hexa-, hepta- and octa-CDD and octa-CDF raised on contaminated wood shavings, see Table 6-3.

Rice grain from ice fields in Arkansas, Louisiana and Texas treated at a maximum rate to give 2.25 lb/acre 2,4,5-T were analyzed for possible 2,3,7,8-tetra-CDD residues. A specification of 1 µg 2,3,7,8-tetra-CDD is given in the report, but no analytical data is given for the herbicide. No 2,3,7,8-TCDD was detected in the rice grains with a detection limit of 2-7 pg/g. No 2,3,7,8-TCDD residues (detection limit 2-10 pg/g) were found in 30 samples of rice purchased in retail stores throughout the USA (Jensen et al 1983).

6.5 Daily direct contact

The widespread use of hexachlorophene in soaps, shampoos and cosmetics may result in direct daily contact with hexachlorphene as well as its impurities including TCDD and hexachloroxanthene, at least for a selected population segment.

Chlorinated phenols (including 2,4,5-trichlorophenol) have been widely and variously used: as fod additives; in hide preservation and hide curing; as herbicides and wood preservatives including indoor use, and as slimicides in paper mills (Firestone 1978; Nilsson et al 1978). Since no precautions are taken to minimize the presence of any PCDD or PCDF other than TCDD, the use of chlorophenols could possibly result in a general low background of the stable PCDDs and PCDFs present as impurities.

Occupational exposure as well as TCDD-related skin effects observed in members of the households of workers from TCDD-contaminated plants will be discussed in section 9.

7 CHEMOBIOKINETICS AND METABOLISM

7.1 Uptake, distribution and excretion

7.1.1 Respiratory tract

No studies on the uptake of TCDD from the respiratory tract have yet been reported.

7.1.2 Skin

Poiger and Schlatter (1980) studied the dermal absorption of ^{3}H-TCDD (specific activity 46.4 Ci/mmol) in hairless rats of the Naked ex Back-Cross and Holzman strain (200-250 g). They also compared the results from the dermal absorption to the uptake of TCDD after an oral administration, and investigated the effect of different solvents and adsorbents on the uptake of radioactivity. Using the amount of TCDD-derived radioactivity found in the liver as an indicator of its absorption, they discovered that the permeation of TCDD across the epidermis was highly dependent on the formulation used. The highest radioactivity in the liver, 14.8 ± 2.6 per cent of the administered dose, was detected when TCDD was applied in methanolic solution. Adsorption of the compound onto activated carbon completely prevented its percutaneous uptake.

7.1.3 Gastrointestinal

7.1.3.1 Studies on rats

Piper et al (1973) gave male Sprague-Dawley rats ^{14}C-TCDD by gavage in a dose of 50 µg/kg body weight. In a group of 3 rats kept in metabolism chambers approximately 30% of the administered dose of radioactivity was eliminated in the feces during the first 48 hours. Most of this probably represented unabsorbed TCDD. For the next 19 days, only 1 to 2% of the dose appeared in the feces each day. The total fecal content was 53.2% of the dose over a 21-day period. During this time 13.2% was excreted 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 experiment, groups of 2 to 3 rats were killed at different time intervals after similar administration of the same dose of ^{14}C-TCDD. 3, 7 and 21 days after dosing, the liver contained, respectively, 3.2, 4.5 and 1.3% of the administered dose per gram of tissue. 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) Sprague-Dawley male rats were given ^{14}C-TCDD in a single dose of 50 µg/kg body weight by stomach tube. Groups of 5 rats were sacrificed 1, 3, 5, 7, 14 and 21 days after dosing. Another 10 rats were followed until the death of half the animals 25 days after dosing. 25% of the dose was eliminated within the first 3 days through the feces. During the subsequent 18 days between 1 and 2% of the dose was found in the feces daily. The total amount in the feces during the 21 days following administration was about 52%. The authors concluded that the fecal 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 gastro-intestinal 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-activity excreted through the urine over a 21-day period was about 4.5% of the dose. On the basis of the daily fecal 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 and Marrow (1975) fed Sprague-Dawley male and female rats a diet containing 7 or 20, yg ^{14}C-TCDD/kg feed. Two animals of each sex and TCDD dietary level were sacrified at 14-day intervals. Most of the TCDD was found in the liver of both sexes with a gradual increase of the ^{14}C-content reaching in males, at the end of the 42-day feeding period, the ^{14}C levels corresponding to a TCDD equivalent of 5.8 and 15.9 yg/kg of liver for the lower and higher feed concentrations respectively. The concentrations in the liver of female rats were similar. Analyses of the liver of rats killed 14 and 20 days after discontinuing TCDD exposure indicated a gradual decrease in TCDD liver concentration in both sexes, the values, at these time intervals, for males exposed to the lower TCDD feed level being 3.8 and 1.0 yg/kg and for those exposed to the higher TCDD feed level 5.8 and 3.3 yg/kg.

Rose et al (1976) studied the fate of ^{14}C-TCDD in Sprague-Dawley rats given single or repeated, doses of ^{14}C-TCDD by gavage. In the single-dose experiment, urine, feces and expired $^{14}CO_2$ were collected individually for 22 days from 3 male and 3 female rats given ^{14}C-TCDD in a dose of 1 yg/kg body weight by gavage. ^{14}C-activity was detected in the feces but not in the urine or the exhaled air. When the sum of radioactivity excreted and found in the body at the end of the experiment was compared with the administered dose, the overall recovery was 97 ± 8%. When the cumulative percentage of the dose excreted was substracted from 100% for each day, the calculated percentage of the dose remaining in the body as a function of time followed apparent first order kinetics. The calculated mean value of the fraction of the dose reabsorbed by each of the 6 rats averaged 0.84, and the estimated half-time was 31 days. When these animals were killed 22 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. In the experiment with repeated ^{14}C-TCDD administration, 9 adult male and 9 adult female rats were divided into groups of 3 rats per sex per dose and given 0.01, 0.1 or 1.0 yg ^{14}C-TCDD/kg body weight/day, by gavage for 7 weeks. ^{14}C-activity was excreted primarily in the feces, but a significant amount was also found in the urine. The percentage of the dose excreted in the urine compared with that excreted in the feces tended to increase with time. In rats given repeated doses of 1.0 yg/kg body weight/day, males excreted 3.1 ± 0.2% and females 12.5 ± 5.1% of the cumulative dose in the urine within 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 studied in these experiments. After 7 weeks' exposure the calculated average body burdens were 48.3 ± 3.9 and 41.0 ± 4.3% of the administered dose for the rats given 0.1 and 1.0 µg/TCDD/kg/day, whereas the directly determined

values were 47.7 ± 8.8 and 37.1 ± 7.5% of the dose. The best estimates for the fraction of the dose absorbed (f) and for the rate constant of elimination (JO during the 7-week exposure period were obtained by fitting the body burden versus time data for each rat to s one-compartment open model exhibiting an apparent first order rate of elimination (Table 7-1). Pooling the results from all rats, the overall rate constant for elimination corresponded to a half-time of 23.7 days. Using these data the authors concluded that:

i. the ultimate steady state body burden would be 21.3 D_o for rats given a daily dose D_o, 5 consecutive days per week for an infinite number of weeks;

ii. at the end of the experiment (after 7 weeks of exposure) 76.2% of the ultimate steady state burden was reached;

iii. the time required to reach 902 of the ultimate steady state body burden would be 78.9 days.

Using the same model, the authors calculated that, if the same dose was given each day instead of 5 consecutive days of the week, the same time of 78.5 days would be needed to achieve 90% of the ultimate steady state body burden. However, the body burden achieved at that time would be 26.1 D_o instead of 19.2 D_o.

The ^{14}C-activities determined in the tissues of rats killed after 1, 3 and 7 weeks' dosing expressed as TCDD content assuming that all activity detected is present as ^{14}C-TCDD are presented in table 7-6. When liver samples from rats given repeated doses of 1 yg ^{14}C-TCDD/kg body weight/day for 7 weeks were analysed for TCDD content by gas chromatography and gas chromatography-mass spectrometry, the results of direct chemical determination were not statistically different from concentrations calculated from ^{14}C measurements.

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 the level of 324 ± 53 yg/kg wet weight in males and 284 ± 21 yg/kg wet weight in females given repeated TCDD doses of 1 µg/kg body weight/day. For the dose level of 0.1 yg/kg body weight/day, the liver TCDD levels were 36 ± 4 and 35 ± 4 yg/kg wet weight for males and females respectively. A dose-rate of 0.01 yg/kg body weight/day resulted in TCDD liver levels of 2.6 ± 0.6 yg/kg wet weight in males and 3.7 ± 0.4 yg/kg wet weight in females.

After feeding diets with TCDD levels corresponding to daily dietary intakes of 0.1, 0.01 or 0.001 yg/kg body weight for 2 years, the average concentrations of TCDD found in the liver of female Sprague-Dawley rats were 24.0, 5.1 and 0.45 yg/kg wet weight respectively. The corresponding levels in adipose tissue were 8.1, 1.7 and 0.54 yg/kg wet weight. The intercomparison 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 levels off. This conclusion accords 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 absorption after an oral administration of 14.7 ng ^{3}H-TCDD in 50% ethanol to female Sprague-Dawley rats was about double when compared to that after a dermal application (Poiger and Schlatter 1980), but was almost completely prevented when given in an aqueous suspension of activated carbon. The authors suggest that the extremely strong adsorption of TCDD onto activated carbon might be used in decontamination procedures.

7.1.3.2 Studies on mice

In the experiments by Vinopal and Casida (1973), male white mice (20 g) were given tritium-labelled TCDD in a single dose of 130 µg/kg body weight intraperitoneally. In one mouse, 3 days after TCDD administration, 13% of the administered ^{3}H-activity was recovered in the feces and 0.3% in the urine, 32% was found in the liver and 0.3% in the kidneys. In another experiment, groups of 2 to 6 mice were killed at various time intervals after similar treatment with the same dose of ^{3}H-TCDD. 1 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%. On the 4th, 8th and 11th days the highest amount of ^{3}H-activity was found in the liver in the microsomal fraction, somewhat lower activity being detected in the mitochondrial fraction and the nuclei. The supernatant fraction was practically devoid of any labelled compound. 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) reported the effect of adding different substances to food on the persistence of TCDD in the liver of male C57B1/6J mice. Feeding chow mixed with charcoal was able to reduce the liver content of ^{3}H-TCDD significantly, in some cases to 36% of that of control value. A slight reduction was found also with the addition of 0.5% cholic acid. The toxicity of TCDD was also reported to be prevented in mice fed with charcoal and cholic acid.
The metabolic fate of a single p.o. dose of 135 V9 ^{14}C-TCDD/kg was studied in male ICR/Ha Swiss mice (Koshakji et al 1984). Of the total ^{14}C-activity excreated in the feces during the first 24 h 62 ± 4% represented unchanged TCDD. The authors suggest this to be due to TCDD that was not absorbed, and the

suggestion is supported by the absence of major metabolites in the acetone extract of the fecal material during the first 24 hours. The absorption from the gastrointestinal tract after a single dose of 650 µg ^{3}H-TCDD/kg to the hamster has been reported to be about 73.5% (Olson et al 1980) and in the study of Allen et al (1975) the absorbed dose from the gastro-intestinal tract was calculated to be over 75% of the administered of 50 µg ^{14}C-TCDD/kg in the rat. It is not plausible that there would be such a species difference between hamster and rat on the one side and mouse on the other as regard the gastrointestinal absorption, but the explanation will rather be the different solvent used in the latter study (Koshakji et al 1984). TCDO is usually dissolved in corn oil (Allen et al 1975) or in olive oil (Olson et al 1980) for oral treatment of animals. However, the solvent used in the study of Koshakji et al (1984) contained 1% ethanol (95%), 10% Tween 80, and 89% saline.
The half-life calculated for TCDD in the study by Koshakji et al (1984) was appoximately 20 days which is in good agreement with the studies of other investigators (Piper et al 1973, Rose et al 1976, Allen et al 1975).
After the first 24 h the elimination of the remaining 28% of the administered dose followed apparent first-order kinetics, which has also been reported by others (Piper et al 1973, Rose et al 1976). Besides unchanged TCDD, there were several other components in ether extracts of urine and fecal samples collected jover a 11-day period. These metabolites were not identified, but when the material extracted by acetone from urine or feces of days 2 to 11 was treated with β-glucuronidase, a substance with an R_f similar to one of the components in ether extracts was liberated. This substance accounted for 0.4% of the administered dose. Sulfatase incubation did not change the TLC behaviour of the extracted material.

7.1.3.3 Studies on guinea pigs

Tissue distribution and excretion of TCOD in guinea pigs was studied by Gasiewicz and Neal (1979). The biological half-life of a single intraperitoneal dose of 0.5 yg ^{3}H -TCDD/kg was calculated to be 30.2 + 5.8 days. The value is similar to the rate of ^{14}C-excretion observed in rats (Rose et al. 1976), so it was concluded that differences in rates of excretion of TCDD do not explain the differences in the species susceptibility to TCOD toxicity. Tissue distribution of ^{14}C -TCDD after a single intraperitoneal administration of 2.0 yg/kg during the first 15 days is given in Table 7-3. The highest concentrations of radio-

activity by day 3 to 15 were detected in liver and skin. The highest levels of radioactivity as regards to the subcellular distribution were found in the crude nuclear and microsomal fractions (Table 7-4).

The bioavailability of TCDD in soil was studied in young guinea pigs after an intragastric administration of environmentally contaminated soil samples (McConnell et al. 1984). Groups of six animals each were given soil samples corresponding to dosages of approximately 1, 3 or 10 µg TCOD per kg body weight. The dosages 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 ppb TCDD, respectively. Controls received soil samples, in which no TCDD, PCB's, or PCDF's 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 yg/kg for TCDD in corn oil, 7.15 yg/kg for Times Beach soil, and 5.50 yg/kg for the Minker Stont 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 (Table 7-5).

7.1.3.4 Studies on hamsters

Tissue distribution, excretion, and metabolism of TCDD studied in male Golden Syrian hamster by Olson et al (1980). Following a single oral or intraperitoneal dose of 650 yg ^{3}H- o ^{14}C-TCDD/k body weight the greatest content of radioactivity was recorded in the liver, adipose tissue, and adrenals (Table 7-6). Thus the tissue distribution in the hamster seems to be similar to that reported for guinea pig and rat (Table 7-10). The highest concentrations of ^{3}H in the liver adipose tissue and in the testis were recorded at day 3 after the TCDD-treatment, whereas the highest level in the adrenals, skin and in the thymus was measured at day 1 after the treatment. Urinary elimination of radioactivity was considerably higher in hamsters than in TCDD-treated rats or guinea pigs. The results suggest that only water-soluble metabolites of TCDD are excreted by the kidneys and liver.

There were no significant differences between the half-life of orally or intraperitoneally administered TCDD. The half-lifes of 11 days for the i.p. and 15 days for the oral administration are considerably lower than the half-life of 31 and 30 days reported for the rat and guinea pig, respectively. However, the two-to threefold increase in the rate of TCDD elimination by the hamster does not adequately explain the 100- to 1000-fold greater resistance to TCDD toxicity as compared to the rat and guinea pig.

7.1.3.5 Studies on monkeys

Van Miller et al. (1976) gave 3 adult female rhesus monkeys, A male infant monkeys and 5 adult male Sprague-Dawley rats a single intraperitoneal dose of 400 µg ^{3}H-TCDD/kg body weight in corn oil. Over a 7-day period the adult monkeys excreted 1.06% of the dose in the urine and 3.75% in the feces. During the same period the infant monkeys excreted approximately 2% of the administered dose in urine and about 1.26% in the feces. The authors questioned the accuracy of these figures owing to difficult urino-fecal separation when working with infant monkeys. In male Sprague-Dawley rats, 0.5% was excreted through the urine and 5% through the feces during the same period. Seven days after administration, the total liver content was 10.4± 6.9% of the administered dose in adult monkeys, 4.51±1.60% in infant monkeys, and 43.0±4.7% in rats. Evaluating species differences in tissue concentrations in a number of organs, the authors expressed the results in % of the administered dose/g tissue and found that 7 days after dosing 0.09% of the radioactivity was present per g liver in adult monkeys, 0.13% in infant monkeys, and 4.5% in rats. At this time the adult monkeys stored 0.16% of the dose per gram adipose tissue, the infant monkeys O.A9% and the rats 3.46%. The skin of the adult monkeys contained 0.03% of the administered dose/g in comparison with 0.24%/g in infant monkeys, and 0.13% in rats. However, it should be recognized that this way of expressing results could lead to erroneous conclusions regarding species differences in TCDD tissue concentrations. Owing to differences in body weight, the mass of TCDD given to each animal, and accordingly the mass corresponding to 1% of the dose, was approximately 25 times higher in the case of adult monkeys and 5 times higher in the case of infant monkeys than that given to the rats. Thus, assuming that all the ^{14}C-activity in the tissues was in the form of ^{14}C-TCDD (of the same specific activity as administered), the corresponding TCDO equivalent mass concentration in adult monkeys, infant monkeys and rats would be approximately 1.8, 0.5 and 3.6 mg/kg for the liver; 3.2, 2.0 and 2.8 mg/kg for adipose tissue and 0.6, 1.0 and 0.1 mg/kg for the skin. Disregarding the differences in sex and age of the animals used in this experiment, these results indicate a higher mass concentration of TCDD in the skin of monkeys compared with rats and lower mass concentrations in the liver of monkeys compared with rats given the same single dose of TCDD per kg/body weight.

TCDD may be exceptionally persistent in adipose tissue. Thus, in a preliminary study on rhesus monkey (McNulty et al 1982) the estimated halflife of TCDD in the fat was found to be one year.

7.2 Passage of TCDD or its metabolites into fetuses and milk

Transplacental passage of ^{14}C-TCDD was studied by Khera and 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 hours 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 would be present as ^{14}C-TCDD, the following levels 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, fetus 11±3, 15±1 and 16±1. Studies by Moore et al (1973) indicate that the passage of TCOD 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 3 or 1 µg/kg body weight.

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 ppt. The maximum concentration of TCDD in milk in the first experiment was 20.8 ± 6.6 ppt and in the second experiment 19.3 ± 6.6 ppt. After 18 weeks feeding with TCDD the levels had dropped down to 4.2 and 3.6 ppt 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 ppm 2,4,5-T corresponding to TCDD levels of 5, 15, 50, 150 and 500 ppt (Jensen and Hummel 1982). This resulted in levels of TCDD in the excreted milk of below detection limit, 3, 10, 16-22 and 42-89 ppt respectively indicating that about 10-20% of the dose given is excreted in the milk. The levels in cream were about ten times higher.

Nau and Bass (1981) studied the transfer of ^{14}C-TCDD to embryo and fetus in NMRI mice. The animals were given a single dose of 5, 12.5 or 25 µg TCDD/kg by gavage, s.c. or i.p. 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 analyzed for radioactivity. The results were quite similar to those obtained by Khera and Ruddick (1973) in Wistar rat. Placenta and other extrahepatic maternal organs as well as fetal tissues exhibited TCDD levels, which were several orders of magnitude lower than maternal liver concentrations (Table 7-7). TCDD levels in fetal liver was 2 to 4 times higher than those in other fetal organs.

7.3 Metabolic transformation

7.3.1 Metabolism in vivo

Contrary to the earlier reports of Vinopal and Casida (1973), and Ghiasuddin et al. (1975), both Poiger and Schlatter (1979) and Ramsey et al.(1982) have presented evidence for the in vivo biotransformation of TCDD in the rat. Ramsey et al. (1982) demonstrated the presence of at least 8 metabolites of TCDD by liquid chromatography and incubation of bile with β-glucuronidase indicated the presence of glucuronide conjugates of the metabolites. Male Sprague-Dawley rats Mere administered 2,4 or 6 daily p.o. doses of approximately 15 µg ^{14}C-TCDD/kg body weight (Ramsey et al 1982). The bile was collected for 24 h following the last dose. Poiger and Schlatter (1979) incubated similarly the bile or the dialysate with glucuronidase/arylsulphatase, and increased by that measure the dichloromethane extractable radioactivity from 1.5% to 75%. Accordingly, their results further indicate the excretion of TCDD-metabolites in the form of water-soluble conjugates.

Olson et al (1980) demonstrated by means of high-pressure liquid chromatography, one major and several minor metabolites of ^{14}C -TCDD in the urine and bile of Golden Syrian hamster. They found that all of the excreted radioactivity was due to metabolites of TCDD. The results suggest that the hamster metabolizes TCDD at a greater rate than the rat and guinea pig. A one-year-ol Beagle dog was cholecystectomized and a Thomas cannula was implanted about 3 months before the first dose of TCDD (Poiger and Buser 1982). A total dose of 5.4 mg was administered enterally in 4 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 have been identified by combined gas chromatography-mass spectrometry. The proposed metabolic scheme is found in Figure 7-1.

7.3.2 Metabolism in vitro

Recent studies of Olson et al (1981) have shown the metabolism of TCDD in in vitro incubations with isolated rat hepatocytes. The hepatocyte derived metabolites appeared to be similar to those of bile. Incubation of both in vitro and in vivo metabolites with B-glucuronidase gave results which implied that one or more metabolites may exist in the form of a glucuronide conjugate.

Even if there has been evidence about different metabolites of TCDD since 1979, and the possibility of its metabolic transformation has even been suggested earlier (Allen et al. 1975, Rose et al 1976), no metabolites have been identified until 1982 by Sawahata et al. TCDD was incubated with isolated rat hepatocytes at 37°C for 8 hours, and the resulting incubation mixture subjected to HPLC. The major peak of radioactivity not corresponding to TCDD was incubated with β-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 limited amount of material the identity of these metabolites was not confirmed by gas chromatography mass spectrometry. The two other metabolites of TCDD, 1-hydroxy-2,3,7,8-tetrachlorodibenzo-p-dioxin and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin, which were isolated by means of HPLC, and also identified by mass spectrometry.

7.3.3 Toxicity of metabolites

Not only has identification of several in vivo-metabolites been acchieved, but also the acute oral toxicity of TCDD-metabolites, excreted in the bile of dogs, has been studied in the guinea pig (Weber et al. 1982). ^{3}H-labelled TCDD was administered directly into the duodenal lumen of two 1-year-old Beagle dogs. The administration was done in 4 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 bulked 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 of volumes not exceeding 5 ml of solution/k. Male Pirbright guinea pigs were used in 5 week toxicity study. Five animals in each of 4 dose-groups were administered 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 application. One death in the high-dose group was found to be caused by an accidental gastric perforation. The other animals which died exhibited histological lesions which were explained by authors to be due to material coextracted from bile together with the metabolites. The light microscropic 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 dog are at least 100 times less toxic to male guinea pigs than TCDD itself.

8 EFFECTS ON ANIMALS

8.1 Acute toxicity

8.1.1 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

Studies aimed at determine single lethal dose values for TCDD in various species are summarized in table 8-1. The range of doses required to cause death varies extensively with species and strains of species, as well as with sex, age and route of administration within a single strain.

More than an 8000-fold difference exist 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 in male Golden Syrian hamsters (Henck et al 1981). The rat seems to be the second most sensitive species although there is a more than 10-fold variability in LD_{50}-values between different strains. The oral LD_{50}-value was 22 yg TCDD/kg body weight for male Sherman rats (Schwetz et al 1973) whereas Walden and Shiller (1985) found LD_{50}-values ranging from 164 to 340 yg TCDD/kg body weight when male Fisher 334 N rats from three different suppliers were tested.

Monkeys (McConnell et al 1978b), rabbits (Schwetz et al 1973) and mice (Jones and Greig 1975, McConnell et al 1978b, Smith et al 1981, Vos et al 1974) had oral LD_{50}-values of 70, 115 and 114-284 yg TCDD/kg body weight respectively. Male Sherman rats were 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.

Acute toxic data considering the effect of age at exposure to TCDD are scarce and comparisons are hampered by the absence or uncomplete 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 adults.

Schwetz et al (1973) found LD_{50}-values in rabbits of 115 yg TCDD/kg after oral exposure as compared to 275 yg TCDD/kg after dermal exposure. C57Bl/6-mice seem to be more sensitive to i.p. administration of TCDD (Gasiewicz et al 1983) than to oral administration (McConnell et al 1978b). The LD_{50}-value in guinea pigs was increased from 2.5 yg/kg to 19 yg/kg when the vehicle for the oral administration of TCDD was changed from corn oil to methyl cellulose (Silkworth et al 1982).
Despite equal routes and similar vehicles for the administration of TCDD to Golden Syrian hamsters LD_{50}-values varied between 1157 yg/kg (Olson et al 1980b) and 5051 yg/kg (Henck et al 1981). A possible explanation for this difference could be the spontaneous occurrence of ileitis observed in the study by Olson et al (1980b), which might have increased the susceptibility of those hamsters to TCDD-toxicity.

TCDD affect 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 subhuman primates. Generally it is not possible to specify a single organ whose dysfunction is responsible for death. Overall TCDD seems to have a predeliction to cause pathological changes in epithelial tissues, both cutaneous and internal. This is particularly apparent in non-human primates and is noteworthy in this species in that the leisons mimic to some degree what is observed in humans. The histopathologic alterations in epithelial tissues include hyperplastic and/or metaplastic alterations as well as hypoplastic responses. The toxic responses of various species to TCDD are summarized in table 8-2 adapted from Poland and Knutson (1982).

In all animal species death occured 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 8-1).

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 occured. This TCDD-induced wasting syndrome has been thoroughly investigated in serveral studies further discussed in section 8.4.3.

Severe thymus atrophy was also found at autopsy in all animal species given lethal doses of TCDD. Histologic examinations revealed lymphoid cell depletion in thymus cortex, spleen and lymphnodes. These consistent findings in TCDD poisoning will be discussed in detail in section 8.4.2 together with the other lymphoid tissue-related effects.

The greatest difference between species at necropsy both in the gross and histologic effects, concern pathologic alterations in the liver. As discussed in detail in section 8.4.1, a dose of TCDD, lethal to guinea pigs did not result in liver damage comparable to the liver leisons 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. Neither in the hamster does manifest Liver leisons occur even after fatal doses.

There are substantial interspecies differences in the effects observed also in other organs of animals given lethal doses of TCDD. Icterus was reported in rats (Buu-Hoi et al 1972b, Gupta et al 1973), hepatic porphyria occured in mice and rats (see 8.4.5.4), ascites with subcutaneous edema and hydrothorax appeared in mice (Vos et al 1974, Jones and Greig 1975) and monkeys (Allen et al 1977). Accumulation of serouse fluid in the pericardial sac occured in chickens after a single lethal dose of TCDD (see 8.1.4). Hemorrhages 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 hemorrhages (Vos et al 1974).
Testicular atrophy and degeneration characterized by loss of seminiferous tubules has been observed in mice (McConnell et al 1978), rats (Kociba et al 1976), guinea pigs (McConnell et al 1978) and monkeys (Norback and Allen 1973). Proliferative leisons of the gastrointestinal tract has been found primarily in subhuman primates (Allen and Norback 1977, McConnell et al 1978) whereas proliferative changes of the transitional epithelium in the urinary tract have been found in guinea pigs (Gupta et al 1973, McConnell et al 178) and monkeys (Norback and Allen 1973).
Leisons of the adrenal glands have been observed in mice and guinea pigs (McConnell et al 1978) but only in animals that die.
Acute exposure to lethal doses of TCDD only produce minor hematologic alterations in all species studied. Anemia was not observed in mice or guinea pigs (Zinkl et al 1973) but a moderate anemia and leukocytosis occured in rats

(Buu-Hoi et al 1972) and monkeys (McConnell et al 1978a). On the contrary hemoconcentration was observed in rats after exposure to a somewhat Lower but still lethal dose of TCDO (Greig et al 1973, Zinkl et al 1973). Trombocytopenia and clotting abnormalities were observed in rats after acute exposure to lethal levels of TCOO (Weissberg and Zinkl 1973). Hypocellularity of the bone marrow has been found in mice, guinea pigs and monkeys (Allen et al 1977, McConnell et al 1978).

Clinical chemistry changes including serum enzyme activities, serum protein and lipid levels, observed in animals after acute exposure to TCDD primarily reflect damage to other organ systems, mainly to the liver (McConnell et al 1978a, McConnell and Moore 1979, McCullum et al 1978b, Olson et al 1980). Liver related enzyme activities in serum are adversely 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 near to 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 which show little liverdamage. Generally serum triglycerides and free fatty acids are increased while serum cholesterol is decreased after TCDD-exposure, however, marked species differences excist and again these effects seem to be secondary to liver damage. For further details on hyperlipidemia see section 8.4.1.4.

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

Chloracne-like leisons can be induced by topical application and/or systemic administration of TCDD in rabbits, subhuman primates and hairless mice. These leisons are further discussed in section 8.4.4.

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 subhuman primates are of particular interest. Nine female juvenile rhesus monkeys (Macaca mulatta), were divided into 3 groups of 3 and given TCDD in a single oral dose of 0, 70 and 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 on day 12, conjunctivitis and thickening of the Meibomian glands. Subsequently, the eyelashes, 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 3 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 the monkeys that had been given TCDD. Ascites was noticed in 2 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 2 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 grossly extended into the lamina propria.

8.1.2 Other PCDD-congeners

Schwetz et al (1973) reported that no signs of toxicity were observed in 4 male mice and 2 female rats given single oral doses of respectively 2 and 1 g 2,7-DCDD/kg body weight (purity 99.6-99.8%). For OCDD (purity 98.86%) oral doses of 1 g/kg body weight to 5 female rats and 4 g/kg body weight to 4 male mice did not cause any toxic symtoms. A sample containing two different isomers of HCDD (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 9 PCDDs, including TCDD, in C57Bl/6J mice and Hartley guinea-pigs were compared by McConnell et al (1978b) (Table 8-3). The purity of the various isomers tested exceeded 97%. Groups of 8 male mice and of 6 male guinea pigs were used for each dose of any of the tested compounds. 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 in 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 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 edema 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 scatered 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. 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 it was much less expressed in survivors. Similar thymus and splenic changes were found in mice, however, bone marrow atrophy was found only rarely in this species and then it was much less expressed than in guinea pigs. In the guinea-pigs that died, and occasionally in survivors, a marked hyperplasia of the renal pelvis was observed, invariably extended into the ureter and at times involving the urinary bladder mucosa. Gastrointestinal hemorrhages and occasionally microscopic dilatation of crypts in the glandular portion of the gastric mucosa were observed in dead PCDD-treated animals of both species. Retro-orbital hemorrhages with exophthalmus and hemorrhages with detachment of the retina, were seen in mice that died after a lethal dose of any of the congeners tested. Adrenal hemorrhages 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 involvment in all the mice, whether they died or survived, given the same dose. Minimal Liver changes were detected in guinea-pigs, changes largely confined to central congestion with occasional degenerated 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 below the LD_{50}. Hemolysis and hyperproteinemia were found in dying animals of both species. In mice surviving 30 days, but not in guinea-pigs, the blood alpha-globulin level was decreased with a resultant increase in the albumin/globulin ratio. With all PCDDs, for which it was possible to establish the LD_{50}-value, these 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-TCDD or 2,8-DCDD the LD_{50}-values were in the range 1000 to 100 000 times higher than with TCOD. The addition of a chloride atom at an ortho-position, i.e. 1,2,3,7,8-PCDD, 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-HCDD and 1,2,3,6,7,8-HCDD remained comparatively low. The toxicity of 1,2,3,4,6,7,8-HCDD was further reduced. A reduction in the rate of weight gain was observable in guinea pigs given doses of this compound exceeding 200 yg/kg body weight. However, no death was observed during the experiment even at doses as high as 600 yg/kg body weight.

8.1.3 Toxicity in cell cultures

The finding that TCDD at a concentration of 0.1 ng/ml culture medium significantly inhibited all phases of mitosis of dividing endosperm cells of the African Blood lily (Jackson 1972), suggesting that TCDD could be an inhibitor of cell division, prompted several studies on the growth of mammalian cells in culture. 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 respons to TCDD (Beatty et al 1975, Knutson and Poland 1980, 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 to 300 nM concentration of TCDD for 72 h. This dose is to consider high when compared to the LD_{50} in rats and mice. No effect on these cells was observable when exposed to 30 nM.
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 50, 100 or 200 nM TCOD for 48 h (Yang et al 1983).

8.1.4 Chick edema disease

Chick edema disease first gained attention in the United States in 1957. An extensive outbreak among chickens occurred in that year in Georgia (Simpson et al 1959, Sanger et al 1958, Firestone 1973). The cause of chick edema disease was traced to the presence in the feed of toxic components later identified as belonging to the family of chlorinated dibenzo-p-dioxins. TCDD was identified as one of the isomers in the mixture of chlorinated dibenzo-p-dioxins capable of producing chick edema (Flick et al 1973).
Clinical signs of chick edema disease consist of dyspnoea, reduced body weight gain, stunted growth, subcutaneous edema, 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 edema and perivascular lymphocyte infiltration as well as edema of the cardiac muscle with interstitial lymphocytic infiltration (Allen and Lalich 1962, Allen 1964, McCune et al 1962, Simpson et al 1959).
Experimentally chick edema 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 tetra-chlorodibenzo-p-dioxins were fed at dietary concentrations of 0.01 µg/kg the chickens developed edema and 83% of them died (Flick et al 1972).
Potency for inducting chick edema was compared for 3 different PCDDs: TCDD (purity 91% and >99%), HCDD (purity >99%, 2 isomers) and OCDD (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 edema occurred in birds given oral doses of 1 or 10 µg TCDD/kg/day, or of 10 and 100 µg HCDD/kg/day. Chick edema was not observed in chicks maintained on a diet containing 0.1 or 0.5% OCDD.

8.1.5 Toxicity in fish

TCDD is extremely toxic to fish. In static water tests with young coho salmon (Oncorhynchus kisutch) Miller et al (1973) found that 24 to 96 hours of exposure to TCDD (purity 98.7%) added to water in glass containers, 5.4 µg/kg fish body weight (initial water concentration 5.6 ng/l), caused 55% mortality within 60 days. All fish died within 40 days following a dose of TCDD that was 10 times higher. TCDD added to water, 0.054 pg/kg fish body weight (initial water concentration 0.056 ng/l), caused 12% mortality in 60 days. The mortality rate in the controls during that same period was 2%. Even at the higher dose, no deaths occurred during the first 10 days following termination of TCDD exposure. Exposure of guppies (Paecilia reticulatus, Peters) to TCDD for 120 hours was fatal to all fish within 37 days. Even at the lowest water concentration used, 0.1 µg/l, only 39% surviving was noted 20 days after termination of exposure. In salmon and in guppies the appearance of the first sign of toxicity, declining interest in food, was delayed for 5 to 10 days. During the following days skin discoloration and fin necrosis were observed. Salmon developed large fungal growths and guppies erosion of the upper jaw. Depressed body growth and food consumption were observed in young rainbow trout (Salmo gairdineri) fed daily a TCDD-containing ratio (2.3 ng/kg feed dry weight) with fin necrosis appearing after 14 days and death after 33 days of exposure (Miller et al 1973). Juvenile rainbow trout exposed to 10 and 100 ppt TCDD for 96 h showed growth retardation and slight edematous changes. At 100 ppt all fry died within 27 days (Helder 1981). In a bioaccumulation study (Isensee 1978) using a model ecosystem all mosquito fish (Gambusia affinis) died within 2 weeks of exposure to TCDD. The fish body burdens resulting from TCDD water concentration of 2.4 to 3.4 ng/l corresponded to a TCDD concentration of 12 pg/kg fresh weight. High TCDD levels in the daphnids, hemorrhages near the gill and anal areas were observed several days prior to death.

8.2 Subchronic toxicity

Data from the earliest subchronic laboratory study, in which rats and guinea pigs were exposed to daily and/or weekly doses of TCDD, were reported in four separate papers covering general effects (Harris et al 1973), pathology (Gupta et al 1973), hematologic and clinical chemistry changes (Zinkl et al 1973) and immunobiological effects (Vos et al 1973). Female CO rats were given TCDD by gavage in daily doses of 0.1, 1 or 10 yg/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, hemorrhages in various organs, and the depletion of lymphoid organs. Weight gain was also reduced at the daily dose level of 1 yg/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 of 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 occured in the 10 yg TCDD/kg/day group. This group of animals also exhibited increased serum bilirubin levels from day 13 on. These parameters were only slightly affected in rats receiving 1 yg TCOD/kg/day. At all 9 dose levels thrombocytopenia occurred. After 3 days of treatment the high and middle dose animals had depressed platelets counts that remained low throughout the study. In the lowe-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 hematological study on female CO rats given daily oral doses of 10 yg/kg body weight (Weissberg & Zinkl 1973).

When oral doses of 0.02, 1.0 or 5.0 yg TCDD/kg body weight was given weekly to groups of 10 female CD rats for 6 weeks all the animals survived. However, body weight gain decreased in the 5.0 yg/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 resported as slight at this dose level. No effect on body or thymic weight and no significant histopathological changes were observed in rats given 1 yg/kg body weight or less.
In contrast, all 10 female Hartley guinea pigs that received weekly oral doses of 1 yg TCDD/kg body weight died, or were killed when moribund, between days 24 and 32. They showed weight loss, lymphopenia and depletion of the lymphoid organs, especially of the thymus. Animals that received 8 weekly doses of 0.008, 0.04 or 0.2 yg/kg all survived, though a significant decrease of body and thymic weights were observed at the dose level of 0.2 yg/kg body weight.

Adult male and female Sprague-Dawley rats, in groups of 12, were given 0, 0.001, 0.01, 0.1 and 1.0 yg TCDD/kg body weight by gavage 5 days a week for 13 weeks (Kociba et al 1976). At the end of the treatment period 5 rats of each sex were killed for histopathologic examination. The remaining animals were continued for post-exposure observation. Doses of 1 yg TCDD/kg body weight/day caused 5 deaths in females, with 3 occuring during treatment and 2 post-treatment, and 2 deaths in males, both occuring in the post-treatment period. Decreased body weights and food consumption were found at the two higher dose levels both in males and females. Decreased relative thymus weight and increased relative liver weight occurred only in the high male dosage group but in both the 0.1 and 1.0 yg/kg female groups. Male rats had significantly depressed hematologic values (packec cell colume, RBC count and hemoglobin) in the two high dose groups while these values were normal in all female rats. Gross as well as histologic examination revealec treatment related effects only in the high-dose groups with some minor findings in the 0.1 yg/kg group. Subcutaneous edema, decreased sizes of testes and uteri and a decreased number of corpora lutea were found at necropsy. Histologic findings were limited to lymphoid tissues, liver and epithelial linings. The lymphoid tissues including thymus was depleted of lymphocytes. The liver of both male and female rats showed pleomorphic and multinucleated hepatocytes. Foci of necrosis with focal reticulo-endothelial aggregations in the areas of parenchymal cell necrosis were observed. Hyperplasia of Kupffer cells and an increased amount of a goldenbrown pigment were noted. The hepatic changes were more pronounced near the periphery of the lobules. Slight hyperplasia of bile ducts and ductular epithelium were present. The uterus was lined by cuboidal epithelium in the

female rats. The rats given 0.01 yg TCOD/kg did not differ in any of these parameters from the controls except for a slight increase in the mean liver to body weight ratio.

Goldstein et al (1982b) exposed groups of 8 female Sprague-Dawley rats to 16 Meekly oral doses of 0, 0.01, 0.1, 1.0 and 10.0 yg TCDD/kg body weight in a study primarily aimed at investigating TCDD-induced porphyria, which is further discussed in section 8.4.5.4. All animals dosed with the high dose died or were killed moribund after eight to twelve doses, a decrease in weight gain was seen in this group within one weeks treatment. Decreased body weight gain was observed also in the 1.0 yg TCDD/kg/week group but not until several weeks after the start of treatment. Hepatic porphyria was found in 7 of 8 animals receiving weekly doses of 1.0 yg TCDD/kg, in 1 of 8 receiving 0.1 yg/kg/week and in none of the animals receiving 0.01 yg/kg/week or the lethal dose of 10.0 yg/kg/week. Six months recovery from TCDD-exposure, at the level of 1.0 yg/kg/week for 16 weeks, could not reverse this porphyria.

8.3 Chronic toxicity

Chronic toxicity studies performed on laboratory animals exposed to TCDD are summarized in table 8-4. Studies concerning carcinogenesis are presented in section 8.7.

Survival for male Spgrague-Dawley rats in groups of 10, maintained on diets containing 0, 1, 5, 50, 500, 1 000, 5 000, 50 000, 500 000 and 1000 000 ppt of TCDD for 65 weeks was monitored by Van Miller and Allen (1977). At the five highest dose levels all animals died before the study was finalized. The first death in these groups occurred by week 31, 31, 3, 2, and 2 of treatment respectively.
Groups receiving 0.05, 0.5 and 1 ppm in the diet died from acute toxic effects including severe liver necrosis, bile duct hyperplasia and edema, atrophy of spleen and thymus and gastrointestinal hemorrhages.

Groups of 50 male and 50 female Sprague-Dawley rats were fed diets providing daily doses of 0.001, 0.01 and 0.1 yg TCDD/kg body weight for 2 years (Kociba et al 1978, 1979). Control rats, 86 males and 86 females, received diets containing the vehicle alone. 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 pg/kg/day, while no increased mortality was observed in male rats at this dose or in animals receiving doses of 0.01 or 0.001 pg/kg/day. From month 6 to the end of the study the mean body weights of males and females decreased at the highest dose rate and to a lesser degree in females given 0.01 pg/kg/day. During the middle of the study lower-than-normal body wights were occasionally recorded also in the low-dose group although during the last quarter of the study the body weights were comparable to those of the controls.

Increased urinary coproporphyrin and uroporphyrin were noted in the females but not in the males given TCDD at a dose rate of 0.01 and 0.1 pg/kg/day. Analysis of blood serum collected at terminal necropsy revealed increased enzyme activities related to impaired liver function in female rats given 0.1 pg TCDD/kg/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. Histopathologic 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 low dose rate.

Weekly oral doses of 0, 0.007, 0.7 and 7.0 pg TCDD/kg body weight/week for 1 year resulted in amyloidosis and dermatitis in male Swiss mice (Toth et al 1979). The incidence of these leisons was 0/38, 5/44, 10/44 and 17/43 in the control, low-, medium- and high-dose groups respectively.

In an early study by Allen and Carstens (1967) groups of 4 to 5 rhesus monkeys were fed diets containing 0, 0.125, 0.25, 0.5, 1.0 and 10.0% "toxic fat", shown to be toxic to chickens, until death. The "toxic fat" was later demonstrated to contain various PCDDs including TCDD, 65% by mass (Norback and Allen 1973). The survival time became shorter with the dose 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 as compared with controls were noted. Both clinical and histologic changes near the time of death appeared similar regardless of dose. The monkeys developed subcutaneous edema, progressing from the eyelids and face, ascites and hydropericardium. Characteristic skin changes were observed as well as anemia, leukopenia and hypoproteinemia. Bone marrow was hypoplastic. Centrilobular

necrosis, bile-duct hyperplasia and multinucleated hepatocytes were found in the Liver. In more than half of the animals marked hypertrophy of the gastric mucosa with crypts and mucin containing cysts penetrating into submucosa and ulcerations in the fundic and pyloric regions were observed.

Allen et al (1977) fed 8 adult female rhesus monkeys a diet containing 500 ppt TCDD for 9 months. Thereafter surviving animals were removed from the TCDD-diet and were observed for another A months. No control animals were included thus data were compared with pre-exposure values where possible. During the first 3 months of exposure animals developed periorbital edema, acne and loss of facial hair and eyelashes. By 6 months these changes were quite prominent in 6 out of 8 monkeys, and a decrease in hemoglobin hematocrit 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 within another 3 months on TCDD-free diet. The three surviving animals experienced a continuing loss of hair and periorbital edema. The total intake of TCDD over the 9 month period was calculated as *2* to 3 µg/kg body weight. Death was preceded by severe anemia, a decreased WBC and severe thrombocytopenia. Autopsy findings included hemorrhage into *a* variety of organs, ascites, and subcutaneous edema. Hypertrophy, dilatation, edema and hydropic degeneration of the myocardium was noted in all animals. The biliary ducts showed marked dilatation. Moderate hyperkeratosis of the skin with cystic keratosis of the hair follicles was noted. Hypocellularity of the lymphoid tissue and the bone marrow were observed. The hyperplastic mucus-secreting cells of the gastric epithelium had invaded the submucosa. Ulceration and mucinous cysts were also common in the modified gastric mucosa. Hypertrophy and hyperplasia of the epithelial lining of the biliary system was present. The bronchial epithelium, the salivary glands, bile ducts and pan-creatic ducts showed metaplastic changes. It was concluded that death was attributed to complications from the severe pancytopenia.
Similar though less severe effects were observed in A adult female rhesus monkeys fed a diet of 50 ppt TCDD for 20 months according to an abstract by Schantz et al (1979).

8.4 Effects detected by special studies

8.4.1 Hepatotoxicity

TCDD produce hepatomegaly due to hyperplasia and hypertrophy of parenchymal cells in all species investigated even at sublethal doses, although there is considerable variation in the extent of this leison among species. Other liver leisons are more species specific.
Liver leisons alone cannot explain lethality following TCOD 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 plasmamembrane function, hyperlipidemia and increased regenerative DNA-synthesis.

8.4.1.1 Morphological alterations

In Charles River rats given single oral sublethal doses of TCDD, 5 or 25 µg/kg body weight, a dose-related increase in the hepatic amount of smooth endoplasmatic reticulum (SER) was observed around the periphery of cells, particularly in the areas around bile canaliculi, 3 days after dosing. The effect progressed by days 6 and 9 when increased amount of rough endoplasmatic reticulum (RER) also was present. These changes had almost recovered by day 28 (Fowler et al 1973).
Livers of CD rats given high sublethal doses of TCDD showed transient degenerative changes followed by megalocytosis, regeneration and the occurrance of multinucleated giant hepatocytes (Gupta et al 1973).
The hepatotoxic reaction in rats given lethal doses of TCDD 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 (Gupta et al 1973). These leisons were considered severe enough tp be a contributing factor of death.
Parenchymal cell necrosis was also observed by Greig et al (1973) in Porton rats exposed to an LD_{50} dose of TCDD, 3 weeks after exposure. The necrosis, which was located to the centrilobular zone close to the central vein, progressed with time.

Jones and Butler (1974) further investigated the time-course for the TCDD-induced liver leisons appearing in the centrilobular zone. They confirmed the transient degenerative and inflammatory leisons previously reported (Greig et al 1973, Gupta et al 1973). At the ultrastructural level consistent changes occured 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 occuring 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 occuring in TCDD treated rats might form by coalescence of parenchymal cells.

The effect of TCDD on plasmamembranes demonstrates a specific subcellular site of action, which might be involved in the toxic action of TCDD. This leison was, however, not observed until 2 weeks post-treatment and thus could not explain the immediate effects on e.g. food intake, body weight gain and general health.

The histological findings were accompanied by hyperbilirubinemia, hypercholesterolemia, hyperproteinemia, increased serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase activities further indicating damaged liver function (Greig et al 1973, Zinkl et al 1973).

Liver leisons produced by TCDD are less well studied in other species than the rat.

Extensive liver necrosis appear in rabbits after a lethal dose of TCDD (Bauer 1961, Kimmig and Schultz 1957).

In C57 mice given single oral doses of 100, 150 or 200 µg TCDD/kg (Vos et al 1974) and 250 µg TCDD/kg (Jones and 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 hepatic esterified fatty acids and cholesterol levels. 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 TCDO based on LD_{50} data, show Less severe morphologic alteration in the liver than do other species. No manifest liver lesions at the light microscopic or ultrastructural levels has been found (Gupta et al 1973, McConnell et al 1978, Moore et al 1979, Turner and Collins 1983).

The hamster is very resistant to TCDD toxicity and exhibits no manifest liver damage even after a fatal dose (Henck et al 1981, Olson et al 1980).

8.4.1.2 Hepatic plasmamembrane function

The morphological impairment of hepatic plasmamembranes in centrilobular parenchymal cells of TCDD-treated Porton rats was demonstrated to be preceeded by a loss of ATPase activity, histochemically determined, in these cells (Jones 1975). The effect occured in an area five to six cells deep around the central vein along the canalicular borders 3 days post-treatment, and progressed with time. At the end of the study, day 42 post-treatment, the ATPase activity was completely abolished around the central vein, including the midzonal 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 post-treatment partial restoration of the normal liver architecture as well as the ATPase reaction were evident.

Biochemical studies of isolated hepatic 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^{2+}-ATPase activity was observed only in the high dose group. The Mg^{2+}-ATPase activity, tended to recover by day 40 whereas Na^+,K^+-ATPase activities did not. A pairfeeding 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.

Many physiological homeostatic mechanisms are dependent on proper plasma membrane function and composition. Recently, Matsumura et al (1984) reported that a single i.p. dose of 25 pg TCDD/kg body weight to male SD rats reduced hepatic plasma membrane ATPase-activities by 40% 10 days post-treatment.

δ-Glutamyltranspeptidase-activity, a marker enzyme for putative preneoplastic hepatocytes, was reduced, while protein kinase, both c-AMP stimulated and nonstimulated, was increased. 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 was not affected by this dose.

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

Quantitative changes in protein composition of plasmamembranes, isolated and analyzed by SDS-polyacrylamide gel electrophoresis from male SO rats 10 days after an i.p. injection of 25 yg TCDD/kg body weight has been reported (Brewster et al 1982, Matsumura et al 1984). Some small size proteins, 14 to 30 Kdalton, were completely abolished by TCDD-treatment. The effect was most pronounced 10 to 20 days post-treatment.

8.4.1.3 Biliary excretion

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

The cumulative biliary excretion of indocyanine green (ICG), an organic anion, was dose-dependently decreased by treatment with 5 or 25 yg TCDD/kg body weight in CD rats (Hwang 1973). On the contrary, biliary excretion of the organic anions sulfobromophtalein and phenol-3,6-dibromophtalein was unaffected by treatment with 10 or 25 yg TCDD/kg body weight in Holtzman rat (Yang and Peterson 1977).

Biliary excretion of ouabain, a model compound for neutral nonmetabolized substrats such as estradiol, progesteron and cortisol, in male Holtzman rats was depressed in a dose-related manner by a single oral dose of 10 or 25 yg TCDD/kg body weight (Jones et al 1977). The effect was detectable two days after treatment, reached a peak between 10 and 20 days and slightly recovered by day 40.

When hepatocytes from TCDD-treated (10 yg/kg body weight) male SD 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 was decreased, whereas the uptake of PAEB was unaffected by TCDD (Eaton and Klaassen 1979). The dose of TCDD was very small relative to ouabain thus it is not likely that TCDD exerted its effect by competing with the drug for transport into bile. Thus, 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 plasmamembrane 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 ouabain excretion and liver membrane ATPase activities could be decreased independently. Thus it is not plausible that ATPase are directly responsible for the reduced ouabain excretion.

8.4.1.4 Hyperlipidemia and fatty degeneration

Liver lipid synthesis in Wistar rats, measured as the 1 h incorporation of ^{3}H-acetate, was not affected by TCDD treatment 7 days after exposure to 10 yg TCDD/kg body weight (Cunningham and Williams 1972). To account for the increase in total liver lipids a restricted transport of lipids out of the liver was suggested.
Fisher rats produced a marked fatty liver after a single oral dose of TCDD (Albro et al 1978). At a sublethal dose triglyceride and free fatty acid levels were elevated while cholesteryl esters were decreased. The effect was seen after one day. The gross levels of lipid returned to normal within 2 to 3 weeks, but unnormal lipid deposition pattern were st'ill persistant for at least 2 months. A lethal dose of TCDD resulted in a persistant increase in free fatty acids and cholesteryl esters in the liver, with little change in triglyceride levels.
The high density lipoproteincholesterol level in serum was dose-dependently increased in male SD rats after a single i.p. exposure to 2.5, 5, 10 and 20 yg TCDD/kg body weight (Poli et al 1980). The effect was persistent throughout the 2 months study.

According to Swift et al (1981) hyperlipidemia induced in guinea pigs by 2 µg TCDD/kg body weight is due to increased serum levels of VLDL and LDL, possibly synthesized from free fatty acids mobilized from adipose tissue.

8.4.2 Immunobiological effects

TCDD produce a pronounced atrophy of thymus, spleen and to a lesser extent peripheral lymph nodes of experimental animals. Since the first report on TCDD-induced thymic atrophy (Buu-Hoi et al 1972a) many studies in rats, mice, guinea pigs and monkeys have shown that the thymus is one of the organs most severly affected by TCDD. Leisons in the thymus appear at exposure levels well below those inducing leisons in other organs. Although there is species variation in the degree and severity of other organ effects the effects of TCDD on lymphoid containing tissues is consistent in all species. Further investigations on 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 immune response and the cell-mediated-immune response. Damage to the thymus and to the cell-mediated immune system seems to be rather specific in that it hits at doses considerably lower than those affecting other immune functions (Faith and Luster 1979). Thymic involution is believed to be a direct effect on the gland thus not secondary to factors such as undernutrition (van Logten et al 1980), altered levels of hormones including corticosteroids (van Logten et al 1980, Vos et al 1973), pituitary hormones (Vos et al 1973) and thymosin (Vos et al 1978), zinc deficiency (Vos et al 1978) or a direct cytotoxic effect on lymphocytes (Vos et al 1978). A direct effect of TCDD on thymusanlagen from mice grown in vitro was demonstrated at concentrations as low as 10^{-10}M (Dencker et al 1985). Within the thymus, lymphocytes within the cortex, i.e. the immature T-cells, are more severly affected in TCDD-treated animals than are lymphocytes within the medulla i.e. the mature T-cells. Thus it seems that TCDD impairs the differentiation of thymocytes into immunocompetent T-cells. Recently Greenlec et al (1985) obtained results demonstrating a direct effect of TCDD on thymus epithelial (TE) cells. High levels of Ah-receptors have been found in the thymus (Carlstedt-Duke 1979, Gasiewicz and Rucci 1984a, Mason and Okey 1982) and studies with C57Bl-mice (responsive to TCDD), DBA-mice (non-responsive to TCDD) and hybrid mice from crosses between these strains, B6D2F_1-mice, suggest

with the Ah-locus in these strains of mice (Clark et al 1983, Dencker et al 1985, Nagaratti et al 1984, Poland and Glover 1980, Vecchi et al 1981). The most profound and persistant effect of TCDD on the immune system is found if TCDD is administered during pre- and/or post-natal life.

8.4.2.1 Histopathology

Lymphoid organs, primarily thymus but also spleen and lymph nodes were affected by TCDD over a wide spectrum of dose ranges in adult rats, guinea pigs and mice (Gupta et al 1973, McConnell et al 1978, Vos et al 1973, Vos and Moore 1974). The marked reduction in the size of thymus has been referred to as atrophy (Gupta et al 1973, Vos and Moore 1974), regression (Allen et al 1975) and involution (Kociba et al 1976) though none of these terms clearly represent the pathogenesis of this leison but are more a description of the final event. Toxic effects on thymus appeared in adult guinea pigs, rats and mice exposed to 8 weekly doses of 0.2 yg/kg body weight, 6 weekly doses of 5 yg TCDD/kg body weight and 4 weekly doses of 5 yg TCDD/kg body weight respectively (Vos et al 1973). The thymus from moribund animals or 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 severly 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 1978). 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 karyorrectic debris and prominent phagocytosis was indisputable proof. Since the thymus from guinea pigs surviving the TCDD dose for 30 days was usually normal microscopically (McConnell et al 1978, Vos et al 1973) it seems that thymic necrosis must be an early event in the course of the toxic syndrome. Furthermore, in animals destined to survive, thymic regeneration seem to be rapid.

Depletion of lymphoid cells in spleen, intestinal tract and various lymph nodes observed in guinea pigs, rats and mice (Gupta et al 1973, McConnell et al 1978) was less extensive than in the thymus. The major effect in the spleen of rats is the loss of the T-celldependent areas, namely the periarterial lymphoid sheat and the paracortical areas (Vos and Moore 1974). Depressed immunoglobulin levels were reported for 1 and 4 months old C57Bl/6 mice exposed to 4 and 6 weekly doses respectively of 25 yg TCDD/kg body weight (Vos and Moore 1974). Feeding 10, 20, 50 or 100 ppb of TCDD in the diet dose-dependently depressed the γ-globulinlevel in 7 weeks old Swiss-Webster mice (Hinsdill et al 1980). Sternal bone marrow from guinea pigs that died from TCDD exposure showed pancytopenic hypocellularity (McConnell et al 1978). Vos and Moore (1974) demonstrated a dose-related lymphocyte depletion of thymus cortex, spleen and intestinal lymphnodes in maternally exposed pups of rats and mice. However, no effect on immunoglobulin levels was observed in 25 days old rats maternally exposed (5 yg 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.

8.4.2.2 Humoral-mediated immunity

The humoral-mediated immunity operates by 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 or, 3- and γ-globulinlevels in C57Bl/6 mice given non-toxic doses of TCDD. Effects of TCDD on specific humoral immunity responses in adult animals are summarized in table 8-5. Feeding levels of 10 ppb TCDD 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). The secondary, but not the primary, serum tetanus antitoxin level was decreased in Hartley guinea pigs given 8 weekly doses of 0.2 yg 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 yg 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. On the contrary a dose of 30 yg TCDD/kg body weight was needed to produce a significant antibody response to the thymus-independent antigen type III pneumococcal polysaccaride

(sIII) in the same strain of 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 given as four weekly doses.
Only minor effects on antibody responses have been reported in rodents maternally exposed to TCDD (table 8-6). Single oral doses of 5 µg TCDD to pregnant Fisher-344 N (poor immunologic responder) and Fisher-Wistar rats (good immunologic 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 and Moore 1977, Faith and Luster 1979). Dietary exposure of female Swiss-Webster mice to 2.5 or 5 ppb of TCDD for 4 weeks before mating, throughout gestation and lactation resulted in normal antibody production in the offspring but a decrease in anti-sRBC plaque forming spleen cells.

8.4.2.3 Cell-mediated immunity

Cell-mediated immunity (CMI) operates by specifically sensitized Lymphocytes and is transferred by these cells. Processes included in this system are classical cell-mediated protective immunity, which protect against fungi, bacteria and viruses, delayed type hypersensitivity, ejection of tumors and foreign tissues such as transplants and graft versus host disease. To test CMI-functions a great number of assays, both in vivo and in vitro, has been developed. Besides a reduction in the number of immune competent cells after TCDD-exposure (Gupta et al 1973, Harris et al 1972, Vos et al 1973, Zinkl et al 1973) TCDD has been demonstrated to induce a decreased CMI-response both in adults (table 8-7) and even more in maternally exposed animals (table 8-8). Delayed hypersensitivity response, correlating with decreased host resistance to infectious agents in man, is depressed in rodents exposed to low levels of TCDD (Clark et al 1981, Faith and Luster 1979, Faith and Moore 1977, Hinsdill et al 1980, Sharma et al 1978, Thomas and Hinsdill 1979, Vos et al 1973). TCDD-exposure also adversely affect host susceptibility to bacteria, viruses, tumor-cells and endotoxins (Clark et al 1983, Hinsdill et al 1980, Luster et al 1980, Thigpen et al 1975, Thomas and Hinsdill 1979). 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/6 Sch mice (Vos and

Moore 1974). In the same study, Fisher-344 rats maternally exposed to TCDD showed decreased graft versus host reponse 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 generation of B-lymphocytes and/or T-lymphocytes from TCDD-exposed animals are depressed both in adults (Sharma et al 1978, Vos and Moore 1974) and maternally exposed rodents (Faith and Luster 1979, Faith and Moore 1977, Luster et al 1980, Vos and Moore 1974, Vos et al 1978a) although Thomas and Hinsdill (1979) found no effect on the lymphoproliferative response in offspring from Swiss-Webster mice fed up to 5 ppb of TCDD 4 weeks before mating, throughout gestation and lactation. Depressed lymphoproliferative response is regarded as an extremly sensitive indicator of immunotoxicity rather than a predictor of immune dysfunction. Cytotoxic T-cell generation in response to allogeneic antigens has been demonstrated in male DBA/2, C57Bl/6 and $B6D2F_1$ mice given 4 weekly i.p. injections of 1 ng/kg body weight (Clark et al 1981, 1983). At this dose no effect were seen on delayed hypersensitivity, antibody response, thymus cellularity nor 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 persistant immune suppression TCDD-exposure during ontogenesis of the immune system is a prerequisite. In the initial experiments on the developing immune system Vos and Moore (1974) exposed Fisher-344 rats to 1 µg TCDD/kg body weight on gestationdays 11, 18 and on postnatal days 4, 11 and 18 or to 5 yg 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 demonstrated immune suppression persisted throughout the study i.e. 145 days. The depression of T-cell dependent immune functions appeared to occur without helper cell function being affected (Faith and Moore 1977).

Attempts to study direct effects of TCDD on lymphocytes in vitro was previously hampered by the low solubility of TCDD in physiological buffers (Matsumura and Benezet 1973). Vos and Moore (1974) obtained no lymphoproliferative response in unstimulated, PHA- or ConA-stimulated rat thymus cells and mouse spleen cells when cultured in the presence of up to 20 ng TCDD/ml. Recently, Dencker et al (1985) demonstrated that fetal thymusanlagen cultured in vitro in the presence of TCDD responded similarly to the response occuring in vivo, i.e. with a dose-dependent inhibition of the time-dependent

increase in the number of lymphoid cells ($EC_{50} \delta 10^{-10}$M TCDD). 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 (Green et al 1984, 1985) thus possibly altering early stages in the pathway of thymus dependent T-lymphocyte maturation which require direct contact between precursor T-cells and the thymic reticulum.

8.4.2.4 Macrophage function

The primary pathway of endotoxin-detoxification is thought to be macrophage-dependent thus the increased sensitivity to endotoxin following TCDD-treatment (Thomas and Hinsdill 1979, Vos et al 1978) was suggestive of macrophage-dysfunction. However, the number of peritoneal macrophages as well as their capactiy to mediate cytolytic and cytostatic effects were not adversely affected by single i.p. doses of 1.2, 6 or 30 µg TCDD/kg body weight to male C57Bl/6J mice (Mantovani et al 1980). Neither was the ability of macrophages to reduce nitroblue tetrazodium affected by 4 to 5 weekly oral doses of 50 µg TCDD/kg body weight in Swiss-Webster mice (Vos et al 1978a). Macrophage proliferation in vitro and the ability of macrophages to phagocytize sheep red blood cells were not affected in $B6C3F_1$ progeny pre- and postnatally exposed to TCDD (Luster et al 1980). Thus macrophage function does not appear to be altered by TCDD.

8.4.3 Wasting syndrome

TCDD causes a starvation like or wasting syndrome in several animal species. In young animals or with a sublethal dose in adults this response is manifested as a cessation of weight gain. Animals exposed to near lethal or higher doses characteristically loose weight. Early studies suggested that acute or chronic treatment with TCDD decreased food consumption, however, not enough 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 if malabsorption could explain the wasting syndrome, the transfer of a number of nutrients have been studied with everted intestinal sacs from TCDD-treated rats. A transient increase in the serosal transfer of ^{59}Fe in SD rats was reported by Manis and Kim (1979). Absorption of glucose (Madge 1977, Ball and Chabra 1981) and lipid (McConnell and Shoaf 1981, Shoaf and Shiller 1981) was decreased by TCDD-treatment. The absorption of cobalt, galactose and prolin (Manis and Kim 1977) as well as of D-galactose, L-arginine, L-histidine (Madge et al 1977) and penicillin (Manis and Apap 1979) was reported to be unaffected by TCDD-treatment. The leucin transport was depressed in SD rats 4 h after a single oral dose of 100 yg TCDD/kg (Ball and Chabra 1981) whereas no effect was observed in Fisher rats 7 days after exposure to 80 yg TCDD/kg (Shiller et al 1982). Neal et al (1979) found a normal absorption and intermediary metabolism of glucose, L-alanin and oleate in guinea pigs treated with a single oral dose of 2 yg TCDD/kg body weight.

Apparently there is 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, electrolyt 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 Gąsiewicz et al (1980) fed rats intravenously with total parenteral nutrition (TPN). Rats that had recieved a single i.p. dose of 100 yg TCDD/kg body weight gained weight similarly to their TPN-fed controls but died albeit, 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 which maintained their start 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. Seefeld et al (1984a) argued 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 1980). 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's effort to reduce its weight to a new lower level of regulation. This hypothesis have been tested in several experiments under carefully controlled feeding procedures.

Single oral doses of 0, 5, 15, 25 or 50 yg TCDD/kg body weight caused a dose-dependent increase in food spillage and a dose-dependent decrease in body weight, feed intake, resting and total oxygen consumption as well as spontaneous motoractivity in male SD rats (Seefeld et al 1984a, Seefeld and Peterson 1983). In animals treated with a sublethal dose feed intake and oxygen consumption recovered within 3 weeks post-treatment to levels appropriate for the reduced weight of the animals. After treatment with a lethal dose rats continously lost weight and decreased the oxygen consumption until death occurred. Reduction of feed intake and body weight followed the same dose and time dependency. Rats whose body weights were reduced by food restriction prior to TCDO treatment started to gain weight when they immediately after treatment were allowed to feed ad libitum. They reached the same final weight as did food unrestricted TCDD-treated controls.

Fecal energy loss and digestible energy intake calculated as percentage of feed energy intake was not affected by a single oral dose of 15 or 50 pg TCDD/kg body weight in ad libitum fed male SD rats (Seefeld and Peterson 1984b). Neither when pair-fed controls was compared with, was there an effect of TCDD (unpublished results cited by Seefeld and Peterson 1984).

That TCDD-treated male SD rats not only maintain but also defend their reduced weight level with the same precision as control animals defend their normal weight level was shown in a subsequent paper (Seefeld et al 1984b). A single oral dose of 15 µg TCDD/kg body weight reduced the body weight to 85% of control rats in ad libitum fed animals. From this weight level it was possible, with dietary manipulations, both to raise the TCDD-treated animals weight back to the level of ad libitum fed controls and to reduce it even further without causing death. Pair-feeding experiments showed that pair-fed animals returned to ad libitum feeding, within 4 weeks returned to almost the same body weight as the control group fed ad libitum throughout the experiment, whereas in the TCDD-treated group the body weight remained depressed. With diets made tasty and extra high in calories, with eggnog, or unpalatable, with 0.3% quinine, control and TCDD-treated animals gained or lost weight in a similar way. Using a 90% eggnog diet or food restriction both control and

TCDD-treated animals gained or Lost weight rapidly and to a similar extent. When returned to standard chow, body weight decreased or increased until they were similar to those of their respective control and TCDD reference group. Body fat, protein and water in respectively standard chow fed and eggnog fed control and TCDD-treated rats were similar when calculated as percent of body weight. However, when comparing standard chow fed controls and eggnog fed TCDD rats, which had essentially the same body weights, TCDD-treated rats had significantly more carcass fat and less carcass protein. Thus TCDD-treated rats seem to regulate their body weight in the same fashion as do control rats but at a weight regulation level markedly reduced as compared to controls.
Besides beeing typical signs of TCDD-toxicity, loss of body weight and appetite are also prominent signs of thyroid dysfunction. Since a single oral dose of 25 yg TCDD/kg body weight is known to increase the thyroid weight, the biliary excretion of tyroxine (T_4) as well as serum triiodothyronine (T_3) and thyroid stimulating hormone (THS) in the rat (Bastomsky 1977), TCDD-induced wasting might be secondary to an effect on thyroidea.
Potter et al (1983) showed that young male SD rats recieving a single oral dose of 45 yg TCDD/kg body weight contrary to pair-fed controls with similar body weight gain has significantly less T_4 in serum but similar levels of T_3 one week after treatment. Serum glucose levels was also decreased by TCDD independently of hypophagia whereas the decrease in serum insulin appeared to result from hypophagia since it was seen both in TCDD-treated and pair-fed controls. These results indicate that the effect of TCDD on thyroid hormones can not explain the TCDD-induced decrease in body weight gain.
Recently Rozman et al (1984) investigated the possible role of the thyroid gland and serum thyroxine (T_4) in mediating TCDD-induced wasting in adult male SD rats. Their data suggest that thyroid hormones are involved in TCDD-induced appetite suppression without being the ultimate target for the lethal effect of TCDD.
TCDD-induced wasting has always been accompanied by the loss of adipose tissue. The rate of fat storage is determined by the lipoprotein lipase activity (LPL) which controls the serum level of triglycerides. To relate these observations Matsumura and Brewster (1984) studied the LPL-activity in guinea pigs exposed to a single i.p. dose of 1 yg TCDD/kg body weight. The LPL-activity was decreased to 20% of the value of ad libitum fed controls. The effect was seen after one day and persisted throughout the study (10 days). Oral intubation of glucose, known to stimulate LPL-activity, reversed the depression in pairfed controls but not in TCDD-treated animals. Serum tri-

glycerides was increased to about 250% of control values by TCDD-treatment whereas no effect was seen in pairfed controls. The authors suggest that TCDD irreversibly reduces adipose LPL thus making the animals less capable to adapt to nutritional changes and needs.
An interesting biochemical effect in the aspect of TCDD-induced wasting is the ability of TCDD to decrease hepatic vitamin A storage in rats. 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 and Morgan 1948, Coward 1938, Hayes 1971, Orr and Richards 1934, Patterson et al 1942).

8.4.4 Epidermal effects

Chloracne and associated pathologic changes in the skin are among the most sensitive and wide spread 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 studies have been performed both in vivo and in vitro.

8.4.4.1 Studies in vivo

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 keratinforming 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 3 successive days (Jones and Krizek 1962). Also Schwetz et al (1973) found a dose-dependent acnegenic respons in the rabbit-ear bioassay after repeated applications of 4 ng to 40 µg TCDD/ear

five days a week for four weeks, corresponding to a total dose of 80 ng to 800 µg. No response was obtained when the total application was 8 ng. Poiger and Schlatter (1980) applied a single dose of TCDD on the inner surface of the rabbit ear and followed the appearance of signs of inflammation, hyperkeratosis and chloracne. The minimum dose that induced skin lesions was around 1 yg TCDD/ear.

Hairless mice constitute an other in vivo modell for studies of epidermal effects of TCDD. Puhvel et al (1982) studied cutaneous changes induced by topical application of 0.1 yg TCDD three times a 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 buildup in the dermal cysts developed in both strains of mice. Follicular keratosis, considered the pathognomonic leison in human chloracne, did not appear within 4 weeks application. In the same study follicular keratosis did develop after topical application of 2 mg 3,4,3',4'-tetrachlorobiphenyl five times a week for 8 weeks suggesting that follicular keratosis is an extension of the epidermal response, thus not related to metabolic changes in sebaceous glands. The authors consider hairless mice a less sensitive model for chloracnegenic response than the rabbit ear bioassay.

Similar findigs were obtained when HRS/J mice were exposed to TCDD topically applied two to three times a week for four weeks (Knutson and Poland 1982, Poland and Knutson 1982). They found a moderate to severe respons, 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, with a total applied dose of 1.2 µg TCDD.

TCDD has been reported to oppose the stimulatory effect of partial hepatectomy on ornithine decarboxylase (ODC) activity, the marker enzyme for proliferative activity (Potter et al 1982). TCDD required a certain amount of time following administration to produce the inhibitory effect. Thus the authors suggested altered responsiveness of the liver to hormonal change more plausible than direct inhibition by TCDD.

8.4.4.2 Studies in vitro

Keratinocytes, the principal cell type of epidermis, comprise an in vitro model for studies of TCDD-induced hyperkeratosis both in human- and animal-derived cell cultures. The respons is analogous to hyperkeratinization in vivo. Newly confluent epidermal cell cultures exhibit proliferative properties while the number of basal cells tend to decrease with increasing time of growth post-confluency. 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 in vitro keratinization response 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 and Poland 1980). 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 choleratoxin, epidermal growth factor or 12-0-tetradecanoylphorbol-13-acetate, other compounds known to affect cellproliferation in XB cells (Knutson and 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^{-11} to 10^{-8} M resulted in normal growth until confluency was reached by day 7, thereafter TCDD-treated cultures showed a persistant decrease in cell growth and cell proliferation as well as changed morphology whereas the viability was intact (Gierthy and Crane 1984). Reseeding these quiescent XBF cells, previously exposed to 10^{-9} M TCDD for 14 days, resulted in normal growth and proliferation as well as susceptibility to TCDD-induced changes in cell growth and morphology. Because XBF cells appears to be a highly transformed variant of XB cells, these cells are inappropriate as a model for TCDD action on normal mammalian epithelial cell proliferation and differentiation.

Recently several continuous lines of human keratinocytes derived from neonatal foreskin (Milestone et al 1984, Osborne et al 1984) or squamous cell carcinomas (SCC (Hudson et al 1983a,b, 1984, Rice and Cline 1984, Willey et al 1984) has been shown to respond to TCDD in nanomolar concentrations with a variety of signs indicating alterations in the ordinary differentiation program. Stimulated ^{3}H-thymidine incorporation was seen in post-confluent

human epidermal cells derived from neonatal foreskin after exposure to TCDD (Milestone and LaVigne 1984). Newly confluent human epidermal cells, derived from foreskin responded to 4 days exposure to 10 nM TCDD in the medium with decreased DNA-synthesis, decreased number of proliferating basal cells, increased 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 and Greenlee 1985). The authors suggest that these effects are secondary to an initial alteration by TCDD of a biochemically regulated event. The decrease in small cell number which was dose-dependent with an EC_{50}-value of 2 nM, was also obtained with 2,3,7,8-TCDF but not with 2,4-DCDD.

Proliferation and differentiation of epidermal cells are normally regulated by several growth factors and hormones e.g. the epidermal growth factor (EGF), vitamin A and hydrocortisone.

Mouse hepatoma cells exposed to TCDD for 24 h exhibited 20% inhibition of EGF binding (Karenlampi et al 1983).

It was demonstrated by Hudson et al (1983a, 1984, 1985) that TCDD dose-dependently decreased the specific binding as well as the cellular uptake of EGF in cultures of both normal and malignant human epidermal cells. The EC_{50}-dose for inhibition was 1.8 nM. A similar inhibitory effect was obtained by 2,3,7,8-TCDF while 2,7-DCDD was inactive even at doses 100-fold greater. Maximal inhibition, almost 60%, of EGF-binding in confluent SCC-12F cells exposed to 100 nM TCDD 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 does not compete for EGF-binding sites. Neither did TCDD affect the process of internalizing the EGF.

The addition of 10^{-6} M hydrocortisone to the medium opposed the growth inhibition of SCC cells grown in 10^{-10} M TCDD (Rice and Cline 1984). Hydrocortisone stimulated several aspect of keratinocyte differentiation. These stimulatory effects were abolished in the presence of 10^{-8} M TCDD although TCDD alone had no effect on these paramters. Neither did TCDD affect the hydrocortisone level in the medium.

TCDD and even more hydrocortisone were able to stimulate stratification in SCC cultures held at confluence for extended periods. This effect was opposed by vitamin A (Rice et al 1984).

Similar to TCDD vitamin A supress the stimulation of keratinocyte differentiation by hydrocortisone. On the contrary, vitamin A has no effect on TCDD-induced growth inhibition or its reversal by hydrocortisone (Rice et al 1983, Rice and 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} M of TCDD 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 high in Ca^{2+} although these cells did stratify and differentiate (Puhvel et al 1984).

8.4.5 Biochemical effects

TCDD has been reported to affect a vast number of biochemical parameters. Characteristic for these effects are that they appear within a couple of days after treatment and then remain for weeks, even after very low exposure. So far no one of the biochemical effects has been able to explain the mechanism behind the toxicity of TCDD.

8.4.5.1 Enzyme induction

Primarily, TCDD has been found to increase enzyme activities although observations on enzyme inhibition have also been made. Since the first reports considering enzyme systems as targets for TCDD (Buu-Hoi 1971, 1972b, Greig 1972, Poland and Glover 1973) 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, is the most thoroughly investigated and aryl hydrocarbon hydroxylase (AHH) is the most frequently assayed enzyme in this system.
tCDD has also been reported to affect UDP-glucuronosyltransferases (UDPGT) and glutathion-S-transferases (GT) which are multifunctional enzyme systems involved in conjugating a wide variety of compounds. Thus they play a key role in biotransformation and detoxification of exogenous compounds.

Most studies have been performed with microsomal enzymes but effects of TCDD has also been found 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 phenomena. Time course studies have shown that maximal increase in enzyme activities are reached within 3 to A 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 and Suzaki 1980, Lucier et al 1973, Poland and Glover 1973a). According to Kitchin and Woods (1979) TCDD-induced AHH activity did not reach the normal level until 6 months after exposure to 2 µg/kg body weight.

Hook et al (1975a) found no appearant dependence on age when studying AHH induction in CD rats, 10 to 335 days old at the time for exposure to 25 µg TCDD/kg body weight.

The relative potency of various polychlorinated dibenzo-p-dioxins to induce AHH activity has been investigated by Bradlaw et al (1976, 1980) and Poland et al (1976). They found an apparant 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 unsufficient 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 TCDD. According to Kociba (1981) induction in the rat takes place at as low as 0.0002 yg TCDD/kg body weight. Yet, in the guinea pig, the animal most sensitive to TCDD-toxicity MFO induction is not a clear-cut sign even at lethal doses. Neither in cell cultures is there a correlation between induction of MFO and toxicity. Furthermore, it is known that metabolites of TCDD are less toxic and more readily excreted than the mother compound (see 7.3.3). Thus, TCDD-induced MFO activities rather represent detoxification than increased toxicity.

However, induction of MFO activities might potentiate the toxicity of other foreign compounds, which require metabolic transformation by the MFO system before they can excert 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 and increasing the extent of covalent binding to liver microsomes (Uotila et al 1978, Berry et al 1976, 1977). TCDD applied topically or subcutaneously increased the carcinogenicity of 3-methylcholanthrene CMC) in DBA/2 mice (Kuori et al 1978) but decreased the carcinogenicity of 7,12-dimethylbenz(a)anthracene (DMBA) in CD-1 mice (DiGiovanni et al 1979). The authors suggests that the ability of TCDD to induce the MFO system is, thus increasing both activation of MC to the ultimate carcinogen and inactivation of DMBA, responsible for 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 pathways including, keratinization, steroid metabolism, lipid metabolism, plasmamembrane function and porphyrinmetabolism, are discussed under respective subheading in connection with other effects of TCDD on these processes.
The minute quantities of TCDD required for maximal enzyme induction or suppression, the long duration of the effect as well as 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. Poland et al (1976) studied a hepatic cytosol species that bound TCDD and was suggested to act 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.

8.4.5.1.1 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 (Aitio et al 1978, Hook et al 1975a, Kitchin and Woods 1979, Lucier et al 1973, Madhukar and Matsumura 1981, Poland and Glover 1973a, 1974) and cytochrome b5 (Hook et al 1975a, Lucier et al 1973) as well as the microsomal enzyme activities involved in the oxidative transformation and conjugation of xenobiotica e.g. aniline hydroxylase, arylhydrocarbon hydroxylase (AHH),

biphenyl hydroxylase, 0-deethylase and UDP-glucuronosyltransferase (UDPGT). These enzyme activities have been investigated in a vast number of studies some of them quoted in table 8-9.

Microsomal glutathion-S-transferase (GT) does not respond to TCDD (Aitio and Parkki 1978, Baars et al 1982, Mukhtar et al 1981) but cytosolic GT has been induced both by a single dose of 17 µg TCDD/kg body weight 2 days post-treatment (Manis and Apap 1979) and by near lethal or lethal doses 1 and 6 days after dosing (Baars et al 1981, Hassan et al 1983, Mukhtar et al 1981). Also glutathion reductase was increased while glutathion peroxidase, both total and Se-dependent, and the content of reduced glutathion were reduced by TCDD-treatment (Hassan et al 1983).

The following hepatic enzyme activities involved in drug metabolism has been reported to be unaffected by TCDD-treatment in the rat: N-and O-demethylation (Beatty et al 1978, Hook et al 1975a, Kitchin and Woods 1979, Lucier et al 1973, Madhukar and Matsumura 1981, Poland and Glover 1973a), epoxide hydratase (EH) (Aitio and Parkki 1978), β-glucuronidase (Lucier et al 1973, 1975) and NADPH cytochrome c reductase (Aitio and Parkki 1978, Kitchin and Woods 1979, Madhukar and Matsumura 1981, Poland and Glover 1974). The glucuronid conjugation of bilirubin (Aitio et al 1979), estrone and testosterone (Lucier et al 1975) in liver microsomes from TCDD-treated rats was not different when compared to control rats.

Some hepatic enzyme activities, not belonging to the MFO system affected by TCDD-treatment are aldehydedehydrogenase (Deitrich et al 1978, Lindahl et al 1978) δ-aminolevulinic acid synthetase (see 8.4.5.4), DT-diaphorase (Beatty and Neal 1978), transglutaminase (see 8.4.4), ornithine decarboxylase (see 8.4.4), plasmamembrane ATPases (see 8.4.1.2), porphyrinogen carboxylase (see 8.4.5.4), prostaglandinsynthetase (see 8.4.5.2) and RNA polymerase (Kurl et al 1982).

Prenatal and postnatal(milk) exposure of TCDD, 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 of TCDD via milk and to the activation of an inducing mechanism occuring 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 1975). Administration of 2.5 µg TCDD/kg body weight to pregnant rats on day 17 of gestation increased the AHH-activity, N-hydroxylation and cytochrome P-450 content in the fetal liver on day 20 of gestation (Berry et al 1976).

AHH induction due to TCOD has been reported to occur also in the brain (Hook et al 1975a), kidney (Aitio and Parkki 1978, Hook et al 1975a, Nagayama et aL 1983, Poland and Glover 1973a, Potter et al 1982), lung (Aitio and Parkki 1978, Hook et al 1975a, Nagayama et *al* 1983, Poland and Glover 1973a), prostate (Haaparanta et al 1983, Lee and Suzaki 1980, Nagayama et al 1983), thymus (Nagayama et al 1983) and intestine (Hook et al 1975a, Poland and Glover 1973a), although intestinal AHH-activity was also reported to be unaffected by 17 and 20 µg TCDD/kg (Aitio and Parkki 1978, Manis and Apap 1979). Testicular (Aitio and Parkki 1978, Hook et al 1975a, Poland and Glover 1973a) and adrenal (Guenther et al 1979) AHH-activities were not induced by sublethal TCDD-doses. The O-deethylation activity in kidney, lung and prostate was increased but no effect was seen on the activity in testes or intestine (Aitio and 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 and Parkki (1978) though in their study intestinal UDPGT was not affected. Renal biphenylhydroxylation activity increased after TCDD-treatment. 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 and Suzaki 1981) and mammary gland (Rikans et al 1979) but not in adrenals (Guenther et al 1979). Less testicular cytochrome P-450 was found after a single dose of 25 vg TCDD/kg body weight (Tofilon and Piper 1982). The GSH-tranferase activity was increased in the lung but not in kidney, intestine, testes (Aitio and Parkki 1978) or prostata (Lee and Suzaki 1980). Neither EH nor NADPH cytochrome c reductase were inducible by TCDD in kidney, lung, intestine, testes (Aitio and Parkki 1978), mammary gland (Rikans et al 1979) or prostate (Lee and Suzaki 1980).

8.4.5.1.2 Studies on mice

Enzyme induction studies on mice have been performed mainly with two strains genetically separated at the Ah-locus thus making them responsive, C57, or non-responsive, DBA, to induction of hepatic cytochrome P-450 related enzyme activities by aromatic hydrocarbons, as represented by 3-Methylcholanthrene (3-MC). However, the extra ordinary potency of TCDD for enzym induction revealed increased hepatic cytochrome P-450 content as well as AHH and O-deethylase activities both in C57 and DBA mice after sublethal exposure to

8.4.5.1.4 Studies on rabbits

Studies on rabbit enzymes have been performed on the New Zealand albino strain, exposed to single doses of 10 to 30 µg TCDD/kg body weight 1 to 5 days prior. Both in adults (Johnson and Muller-Eberhard 1977) and in neonatals exposed in utero (Kohli and Goldstein 1981, Norman et al 1978) did TCOD increase the content of cytochrome P-450. It was shown that TCDD induced the formation of immunologically distinct cytochromes P-450 in adult and neonatal liver (Norman et al 1978). Increased cytochrome P-450 also occurs in the kidney but not in the lung (Kohli and Goldstein 1981, Liem et al 1980). Renal and pulmonary cytochrome P-450 reductase, investigated by Liem et al (1977), 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. On the contrary Hook et al (1975a) saw no effect on the AHH activity in the lung and they reported a decrease in hepatic AHH activity. Biphenyl 4-hydroxylase induction was seen both in liver (Johnson et al 1979) and lung (Hook et al 1975a) whereas no effect was seen in the liver and kidney (Hook et al 1975a). Furthermore, Hook et al (1975a) reported no effects on biphenyl-2-hydroxylation and UDP6T activities in liver, kidney nor lung. A decrease in hepatic but not in renal or pulmonary N-demethylation was found by these authors.

8.4.5.1.5 Studies on hamsters

Golden Syrian hamsters are among the animals most resistant to TCDD-induced toxicity. Although the liver is a target tissue, hepatic enzyme induction has barely been studied in this species. When given a lethal dose of TCDD, increased hepatic glutathion-S-transferase and glutationreductase activities were found, but no effects were seen on AHH or glutathionperoxidase activities. Neither were the hepatic level of glutathion or the in vitro lipid peroxidation affected (Hassan et al 1983).

8.4.5.1.6 Studies on chick embryos

Aryl hydrocarbon hydroxylase and δ-aminolevulinic acid synthetase in the chick embryo have been reported to be extremely sensitive to the inductive effects of TCDD (Poland and Glover 1973b,c). Maximal induction occurred with 155 pmoles TCDD/egg. The induction is 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 and Glover 1973c).

8.4.5.1.7 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, primary hepatocytes as well as established and transformed cell lines.

Lymphocyte AHH inducibility has been investigated in mitogen stimulated human lymphocytes from venous blood of healthy volounteers. Kouri et al (1974) found a dose dependent (0, 0.1, 1.0, 10 or 100 ng TCDD/ml medium for 24 h) increase in AHH-activity. The optimal dose was about 10 ng/ml and the greatest inducing power was 2 to 3 times. 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 lymphocyte AHH induction Freedman et al (1979) used the human B-lymphocyte RPMI-1788 cell-line, proven to induce AHH activity without prior activation. The optimal concentration to stimulate AHH activity was determined to be 10 ng/ml medium. Highly variable AHH-inductions, about 3-fold to 28-fold, were obtained by Nagayama et al (1985) in human lympho-blastoid cell lines, derived from peripheral blood of healthy volunteers of both sexes and of variable ages, when exposing the cells to 7.5 ng TCDD/ml medium for 48 hours.

Steward and Byard (1981) treated primary hepatocytes, isolated from SD rats, for 48 h with various doses of TCDD. They found a 2-fold induction of the AHH activity with 3 pg TCDD/10^6 cells. Maximal induction occured with 2.4 ng TCDD/10^6 cells. 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 α-aminoisobutyric acid uptake as well as tyrosinaminotransferase

activity (Yang et al 1983). Treatment of rats with 25 µg 1,3,6,8-TCDD/kg body weight did not affect these parameters. Neither could these effects be demonstrated in primary hepatocytes from control rats when treated with TCDD (50, 100 or 200 nM medium) in vitro for 48 h.

In a study by Niwa et al (1975) the estimated ED_{50} values for AHH induction by TCDD among 11 established cell lines, fetal primary cultures from 5 animal species and cultured human lymphocytes, ranged from 0.04 ng/ml medium in C57 mouse cultures and 0.08 ng/ml in the rat hepatoma H-4-II-E cell line to more than 66 ng/ml in the HTC rat hepatoma cell line. In a similar study, 2- to 650-fold AHH induction was observable in 8 of 22 different cell cultures exposed to 10^{-9}M TCDD for 24 h (Knutson and Poland 1980). 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 and Poland 1980).

When comparing 24 chlorinated dibenzo-p-dioxin analogues TCDD was demonstrated to be the most potent AHH-inducer (Bradlaw et al 1975, 1976, 1980). The study was performed with a rat hepatoma cell culture extremly sensitive to AHH induction, ED_{50} being about 0.5 pg/10^6 cells.

Five human squamous carcinoma cell lines derived from tumors of the epidermis and tongue responded to TCDD with increased O-deethylase activity, the EC_{50} being 10^{-10} to 10^{-9} M (Hudson et al 1983).

8.4.5.2 Endocrine effects

Human exposure to TCDD has resulted in hirsutism and chloracne, symtoms that suggest an alteration in endocrine regulation. Furthermore, chronic exposure to TCDD impairs reproduction in experimental animals possibly by interfering with the estrus cycle (Allen *et* al 1977, Barsotti et al 1979, Kociba et al 1976, Murray et al 1979). The ability of TCDD to mimic the action of natural steroids with respect to being able to perform various steroid like actions has prompted studies on the binding of TCDD to steroid hormone receptors.

Overproduction of glucocorticoids mimic some of the symptoms of TCDD-toxicity e.g. involution of lymphoid tissues, edema 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-dexmethasone, a potent synthetic glucocorticoid, from normal rat cytosol

glucocorticoid receptor even when present in 200-fold molar excess (Neal et al 1979). Furthermore, Poland and Glover (1976) demonstrated that cortisol and synthetic glucocorticoids did not bind to the TCDD receptor. The increased plasma level of corticosterone that was seen in male SD rats 7 and 14 days after single oral dose of 50 yg TCDO/kg body weight was preceded by anorexic response, enzyme induction and morphological alterations. Thus glucocorticosteroids does not seem to be causative in TCOD-toxicity (Neal et al 1979). Neither does adrenalectomy protect against TCDD-toxicity in rats. Neal et al (1979) reported 100% mortality within 6 days in adrenal-ectomized rats given 10, 20, 40 and 80 yg TCDD/kg body weight. Adrenalectomy and hypophysectomy could not prevent liver leisons, reduced growth rate or thymic involution in female Fisher-344 rats exposed to a single oral dose of 10 or 20 lag TCDD/kg body weight (vanLogten et al 1980). Thymic effects of TCDD became even severe after hypophysectomy. Daily s.c. 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.

The finding that steroids may be the endogenous substrats for the hepatic MFO system (Kuntzman et al 1965) suggest that compounds, like TCDD, which influence the activity of this enzyme system would alter the steroid metabolism in vivo and consequently the magnitude of steroid mediated functions. As demonstrated by Gustafsson and Ingelman-Sundberg (1979) the metabolic profiles of 4-androstene-3,17-dione, 5α-androstane-3α,17β-diol and 4-pregnene-3,20-dione in hepatic microsomes from SD rats, treated with 20 yg TCDD/kg body weight for 4 consecutive days, were changed when compared to control rats 1 day post-treatment. Five daily doses of 1 yg TCDD/kg body weight impaired the metabolism of a pharmacological dose of estrogen in Holtzman rats, while physiological estrogen was not affected. Furthermore when the same dose was given to pregnant rats for 12 or 13 days during gestation hepatic microsomes showed decreased ability to form catechol estrogen and to hydroxylate testosterone. However, this decrease did not relate to altered circulating estradiol levels (Shiverick and Muther 1983). TCDD treatment did not affect the glucuronidation of testosterone and estrogen (Lucier et al 1975), prostaglandinsynthesis (Kohli and Goldstein 1981) or hydroxylatior, of testosterone in 23- and 16α-positions (Hook et al 1975b) in hepatic microsoms.

Decreased cytochrome P-450 content was found in guinea pig (Tofilon et al 1980) and rat (Tofilon et al 1982) testes for at least one week after TCDD treatment. Testicular AHH activity was induced by TCDD in mice (Mattison and Thorgeirsson 1978) contrary to the rat (Aitio and Parkki 1978, Poland and

Glover 1973a) which was not affected. Increased AHH and O-deethylase activities as well as cytochrome P-450 content has been reported in rat prostate (Haaparanta et al 1983, Lee and Suzaki 1980).

8.4.5.3 Vitamin A storage

The microscopic appearance of chloracne, the most prominent sign of TCOD induced toxicity in humans, resembles in some respects observations made in the skin of patients suffering from vitamin A deficiency (Kimbrough 1974). Decreased hepatic vitamin A storage has been reported in animals exposed to variuos chlorinated aromatic compounds (table 8-10). As can be seen in table 8-10 TCDD is unique in its ability to reduce the vitamin A content of the liver, both regarding the minute quantities needed to produce this effect and the persistance of the effect.

A single oral dose of 10 yg 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 already 4 days after dosing and progressed with time. After 8 weeks the treated animals had a total liver storage corresponding to 33% of that of controls. Food consumption was measured weekly and the dietary intake of vitamin A was calculated to be about 6 and 5 mg for control and TCDD-treated animals, respectively. Thus it seems unlikely that differences in dietary vitamin A intake could account for the whole difference in hepatic storage.

In a four-week study TCDD wss given as 3 single oral dose of 0, 0.1, 1.0 or 10 yg per kg body weight to adult male Sprague-Dawley rats, fed ad libitum pelleted diets containing 1.2 (low), 3.0 (normal) and 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 diet. In the animals on the normal and Low diets, significant differences were seen only at doses of 1.0 and 10 yg/kg. A significant increase in the UDPGT activity was observed in all dietary groups treated with 1.0 and 10 yg TCDD per kg suggestive of an increased excretion of glucuronide conjugated vitamin A. Homozygous Gunn rats, lacking inducible UDPGT (Aitio et al 1979) and heterozygous Gunn rats, with inducible UDPGT, were treated with a single oral dose of 10 yg TCDD/kg body weight (Thunberg and Håkansson 1983). No correlation between the UDPGT-activity and the reduction of vitamin A in the livers was seen four weeks post-treatment.

Male SD rats receiving a single oral dose of 10 yg TCDD/kg body weight four days prior to the oral administration of a single physiological dose of labelled vitamin A, namely 11,12-^{3}H-Retinylacetate (RA> (H9kansson and Ahlborg 1984, 1985a), handled the newly administered dose of vitamin A in a similar way as do vitamin A deficient rats (Blomhoff et al 1982, Huque 1981). This finding is remarkable since the TCDD-treated animals in this study still had considerable hepatic stores of vitamin A and did not show decreased levels of serum vitamin A i.e. they were not vitamin A deficient.

To elucidate if dietary vitamin A would reduce TCDD-toxicity Hikansson and Ahlborg (1985b) fed male Sprague-Dawley rats ad libitum from weanling throughout the experiment with the following pelleted diets: 2 000 (I), 5 000 (II), 8 000 (III) and 21 000 (IV) IU of vitamin A/kg. A single oral dose of TCDD (15, 30, 60 or 120 yg TCDD/kg body weight) was given when the rats were 8 weeks old. Animals were killed 44 days post-treatment. Body weight gain and relative thymus weights were reduced while relative liver weights were increased dose-dependently in all dietary groups. With diet IV, TCDD reduced hepatic vitamin A dose-dependently. The reduction was from 59 to 90%. With diets II and III hepatic vitamin A reduction was more than 95% by 15 and 30 yg TCDD/kg respectively. In control animals fed diet I total hepatic vitamin A was less than 1 yg 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 rather increased serum vitamin A. Only with the highest TCDD-dose was there a counteraction by dietary vitamin A on all of these parameters.

Taken together these data indicate that TCDD interferes with the storage mechanism for vitamin A. In the liver this mechanism has recently been more thoroughly investigated (Blomhoff et al (1982), Hirosawa and Yamada (1973), Olson and Gunning (1983)). As dietary vitamin A not seems able to counteract the toxic effect, this would imply either that the effect on vitamin A storage is secondary to TCDD-toxicity or that not only storage but also the cellular utilization of vitamin A is affected by TCDD.

8.4.5.4 Porphyria

Chronic sublethal exposure to TCDD produce an accumulation of porphyrins in the liver and an increase in urinary porphyrin excretion. In stages of manifest porphyria, accumulation of porphyrins occur not only in the liver but

also in the kidney and spleen (Goldstein et al 1982). In two early studies it was demonstrated that mice respond to A weekly doses of 25 pg TCOD/kg body weight with hepatic porphyria accompanied by increased δ-aminolevulinic acid synthetase(ALA)-activity and liver lesions (Goldstein et al 1973) whereas a single dose of 5, 25 or 100 µg TCDD/kg body weight could not induce porphyria nor ALA-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/week to rats for 16 weeks resulted in hepatic porphyria (Goldsten 1976, 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 upon the final dose was not enough to reverse the porphyria. Urinary porphyrins and hepatic ALA-activity remained maximally elevated while hepatic porphyrin levels did decrease during this period. Complete recoveries were found for AHH- and UDPGT-activities. 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 depend on that porphyrin-analysis were not satisfactory performed (Goldstein et al 1982).

To further characterize TCDD-induced porphyria Cantoni et al (1981) performed a 45 week time-course study to follow the pattern of porphyrin excretion in rats exposed 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 0.01 dose group. A marked porphyric state appeared only in the 1.0 dose group from 8 months onward. 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 understand 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, the initial and rate-limiting enzyme in heme synthesis, in the liver of chicken embryo (Poland and Glover 1973). Elevated ALA activity has thereafter 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. Jones and Sweeny (1980) failed to demonstrate increased ALA-activity in mice exposed to 25 µg TCDD/kg body weight/week for 11 weeks although porphyria was evident. Thus induction of ALA does not seem to be the primary event in TCDD induced porphyria. Elder

et al (1976) suggested that decreased porphyrinogen carboxylase is the primary event in porphyria induced by halogenated aromatics. It has been demonstrated that TCDD depress this enzyme activity in vivo in the liver of mice (Cantoni et al 1984a,b, Elder and Sheppard 1982, Jones and Sweeny 1980) but not in vitro (Cantoni et al 1984b). The decrease in porphyrinogen decarboxylase activity was present already one week after a single dose of 75 µg/kg body weight and continued to decrease with time, thus preceeding the increase in hepatic porphyrins, which started to rise first 2 weeks after treatment (Smith et al 1981). Thus this study further support the hypothesis that inhibition of porphyrinogen carboxylase is the biochemical mechanism underlying the over-production of porphyrins with high numbers of carboxylgroups (Elder et al 1978, Kushner et al 1976). Recently Greig et al (1984) demonstrated that pretreatment of mice, 5 different strains, with 12.5 mg Fe one week before the administration of 75 µg TCDD/kg body weight had a synergistic effect on porphyria assessed 5 weeks later as increased hepatic porphyrin and decreased porphyrinogen decarboxylase activity. Iron alone did not rise hepatic porphyrin levels nor did it affect hepatic porphyrinogen carboxylase activity.

8.5 Teratogenicity and reproductive effects

Since the initial report by Courtney et al (1970a) on the teratogenic effect of 2,4,5-T, research has been focused on determining the role of TCDD in eliciting teratogenic responses. Studies using purified TCDD are presented in table 8-11.

8.5.1 Studies on rats

The teratogenic effect of TCDD differ from that in the mouse. The results are summarized in table 8-12.

Generally TCDD seems to be more fetotoxic to the rat in comparison to the mouse when considering fetal weight, intestinal hemorrhage and subcutaneous edema. However, other studies performed on the rat have not detected TCDD-related kidney malformations (Giovani et al 1982, Khera and Ruddick 1973, Sparschu et al 1971). Malformations were predominately in form of cystic kidney and dilated renal pelvis for doses above 0.5 µg/kg/day (Courtney and Moore 1971, Giovani et al 1983).

In a 3-generationstudy using Sprague-Dawley rats the animals were maintained on diets providing doses of 0, 0.001, 0.01 or 0.1 yg TCDD/kg/day. Fertility was greatly reduced in the f_0 generation exposed to 0.1 yg TCDO. The group was therefore discontinued because few offspring were produced in this group (Hurray et at 1979).

At 0.01 yg TCDD-dose level body weight was significantly reduced in the f_{1a} and f2 rats. Decrease in litter size were noted in the f_{1a} group at 0.1 yg/kg/day and the f_2 and f_3 litters exposed to 0.01 yg/kg/day. Decreased survival was noted in the 0.01 yg/kg/day group for the f_2 and f_3 litters. Murray et al (1979) concluded that TCDD at the doses 0.01 or 0.1 yg/kg/day impaired reproduction among rats and that the NOEL was at 0.001 yg TCDD/kg/day.

However, these data (Murray et al 1979) have been reevaluated by Nisbeth and Paxton (1982) using another statistical method. They stressed that 0.001 yg/kg/day was not a NOEL in this study.

8.5.2 Studies on mice

The teratogenic effects in mice are rather uniform for mice including cleft palate and kidney malformations.

However, species differences exist which have been shown by Poland and Glover (1980). They have shown that sensitive mice posess high levels of the Ah receptor whereas the resistent mice posess low levels of the Ah-receptor protein. These data are supported by D'Argy et al (1984), Hassoun and Denker (1982), and Pratt et al (1984) thus suggesting that cleft palate formation induced by 2,3,7,8-TCDD coincide with binding to the Ah locus.

The no-effect-level (NOEL) for teratogenic response in the mouse seems to be about 0.1 yg/kg/day based on the study by Smith et al (1976).

8.5.3 Studies on rabbits

New Zealand rabbits were administered TCDD by gavage doses of 0, 0.1, 0.25, 0.5 and 1 yg/kg/day on days 6 to 15 of gestation (Giovani et al 1982). The fetuses were examined on day 28 of gestation.

2/15 and 4/10 maternal deaths respectively.
An increase in abortion and resorption rates occurred at doses above 0.25 yg/kg/day with no live fetuses detected in the 1 yg/kg/day dose group. There was a significant increase in extra ribs from 33.3% in the controls to 82, 66.6 and 82% in the 0.1, 0.25 and 0.5 yg/kg dose groups. There were no increase in specific soft tissue anomalies.

8.5.4 Studies on monkeys

The fetotoxic and teratogen potential of TCDD have been studied in Rhesus monkeys by Allen et al (1977, 1979) and McNulty (1984). Mc Nulty (1984) showed that doses of TCDD given as a single dose or in divided in nine doses are fetotoxic at a total dose of 1 yg/kg body weight which implicates that pregnant rhesus monkeys and their fetuses are very sensitive to TCDD. Minor abnormalities in the palate has been noted at the 1 yg dose level in two of the three living fetus (McNulty 1984). Female rhesus monkeys fed on diets containing 50 to 500 ppt of TCDD which correspond to a total dose of 1.8 and 11.7 yg. At seven months the female were bred with nonexposed males. Of the pregnant monkeys 4/6 and 2/3 had spontaneous abortion at the high dose and low dose respectively. The studies are too limited to reach any conclusion considering the teratogenic potential of TCDD.

8.6 Genotoxic effects

8.6.1 Mutagenicity

8.6.1.1 Studies on bacteria

Contradictory results have been published on the mutagenic effect of TCDD in bacteria.
In two studies by Hussain et al (1972) and Seiler (1973) a strong positive respons was reported in the Salmonella typhimurium strain TA 1532, in a plate test after preincubation of bacteria in medium containing TCDD, and a spot

test respectively. A weak mutagenic respons was also obtained with Escherichia coli Sd-4, measuring reversion to streptomycin independence (Hussain et al 1972). In both studies no metabolic activation systems were present.
Some more recent publications however do not report any mutagenic effect of TCOD in Ames' Salmonella plate incorporation assay including strains TA 1530, TA 1532, TA 1535, TA 1537, TA 1538, TA 98, TA 100, neither in the presence nor in the absence of metabolic activation systems from rat and Syrian hamster (Gilbert et al 1980, Geiger and Neal 1981, Mortelmans 1984). In these studies the earlier reported positive tester strain TA 1532 was either replaced by strain TA 1537 (Geiger and Neal 1931, Mortelmans 1984) or was tested in addition to TA 1537 (Gilbert et al 1980). Strain TA 1537 is a descendant of TA 1532 and is more sensitive by its better uptake of larger molecules. TCOD was tested in the dose-range 0.2 to 2 000 µg/plate. Due to limited solubility of TCDO a maximal dose in the system is reported to be 20 µg TCDD/plate (Geiger and Neal 1981).

8.6.1.2 Studies on eukaryotic cells

Bronzetti et al (1983) reported a mutagenic respons of TCDD in yeast in an in vitro suspension test and an "in vivo" intrasanguineous host mediated assay. In both assays Saccaromyces cerevisiae strain D7 was used. Positive respons was obtained in vitro in presence of metabolic activation at doses up to 10 µg TCDO/mL and in the host mediated assay after treatment of mice with a single dose of TCOD (25 yg/kg).
In L5178Y mouse lymphoma cells TCOD induced mutations in a dose dependent manner at doses of 0.05 to 0.5 yg TCDD/ml where survival of cells at the highest concentration was not lower than 75% (Rogers et al 1982).

8.6.2 Interactions with nuclei acids

Poland and Glover (1979) found that TCDD bound to rat liver nucleic acids at very low levels. The maximum covalently bound TCDD was calculated to 6 and 12 pmole per mole of nucleotide residues from DNA and RNA respectively. Guenther et al (1979) demonstrated in vitro metabolism of TCDD tc reactive inter-

mediates, which bound covalently to cellular macromolecules principally to protein. However, no isomer specific methods were used for analysis of metabolites in this study.

Liver slices from Sprague-Dawley rats treated with 5 yg TCDD incorporated 2 times more thymidine in nuclear DNA 10 days post-treatment than did controls (Conoway and Matsumura 1972). Christian and Peterson (1983) found no effect on the in vivo incorporation of thymidine into liver DNA 35 to 36 h after the administration of 10 yg TCDD/kg body weight to SD rats. Proliferative DNA synthesis, measured as the 1 h in vivo incorporation of thymidine, stimulated by 70% hepatectomy in Porton rats was not affected by treatment with 10 or 200 yg TCDD/kg body weight 0, 24 or 72 h before the hepatectomy was performed (Greig et al 1974). On the contrary Dickins et al (1981) found an 8-fold to 10-fold increase in the DNA-synthesis response to the proliferative signal of 1/3 hepatectomy in Sprague-Dawley rats when 5 yg TCDD/kg body weight was administered 5 days prior to the hepatectomy. In this study tymidine incorporation into DNA was measured in vitro at various times after the 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 Dickens et al (1974) 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 thus making a contributing effect of TCDD difficult to demonstrate. This suggestion was confirmed by Christian and Peterson (1983) who compared the effect of TCDD on proliferative DNA synthesis after 1/3 and 2/3 hepatectomy. This study also revealed that only when a certain amount of time, namely 5 to 10 days, elapsed between TCDD-administration and hepatectomy could the effect be seen. Enhanced incorporation of thymidine was also seen in laparatomiced TCDD-treated rats.

The transfectivity of bacteriophage Qβ/RNA was evaluated after treatment with TCDD. No effect was noticed in the tested dose interval (0.2 to 4 yg TCDD/ml) (Kondorosi et al 1973).

In binding experiment of radiolabelled TCDD to liver macromolecules in Sprague-Dawley rats there were extremely low levels of radioactivity associated with RNA and DNA, close to background (Poland and Glover 1979).

The effect of TCDD on repair of DNA damages introduced by 2-aminofluorene (AF) and 2-acetylaminofluorene (AAF) in primary hepatocytes from B6 and D2 mice have been investigated (Möller et al 1984). By *in vivo* pretreatment with TCDD (50 µM) a slight increase in DNA damage (measured by alkaline elution technique) following incubation with either AF or AAF for 60 min, was noticed.

8.6.3 Cytogenetic effects

Green and Mooreland (1975) and Loprieno et al (1982) did not observe any induction of chromosomal aberrations in rats administered TCDD intraperitoneally or by gavage (5 to 20 µg/kg). In male and female CD-1 mice however a weak but significant respons was obtained when analyzed 96 h post-treatment (i.p. 10 µg TCDD/kg) (Loprieno et al 1982).

In a study on 15 soldiers exposed to Agent Orange no increase in structural chromosomal aberrations or sister chromatid exchanges was noticed when compared to a control group of 8 subjects (Mulcany 1980).

Lymphocytes from inhabitants in Seveso, Italy, accidentally exposed to TCDD in the Seveso accident 1976 were examined for chromosomal aberrations by Regianni (1980) and Mottura et al (1981). In 17 TCDD-exposed individuals examined within two weeks of the accident, no increase of 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, chronical exposure and a control group of non-exposed subjects. No significant difference in frequency of chromosomal aberrations in the three exposure categories was reported. Data on number of subjects, chromosomal aberrations, exposure level and time were not

Tenchini et al (1983) published a comparative cytogenetic study on abortions in women exposed to TCDD after the Seveso accident and nonexposed women. Chromosome analysis was performed both on maternal peripheral blood, placental and umbilical cord tissues and fetal tissues. No significant differences in level of chromosomal aberrations in blood-, placenta-, and umbilical cord-samples from TCDD exposd and nonexposed women was noted. The exception was fetuses samples from nonexposed women where a significant increase in chromosomal aberrations was obtained. A possible artefact due to culturing conditions was discussed. The effect of TCDD on fetal chromosomes is therefore still unclear.

8.6.4 Cell transformation

TCDD has been reported to transform baby hamster kidney cells (BHK) in vitro (Hay 1982). A weaker respons was obtained with the dioxin isomers 2,8-dichloro- and 1,3,7-trichlorodibenzo-p-dioxin. Unchlorinated and fully chlorinated dibenzo-p-dioxin did not transform the BHK cells.

8.7 Carcinogenesis

8.7.1 Long term animal studies

Several studies on the carcinogenicity of TCDD and related compounds have been performed. The data from studies with oral exposure are tabulated in table 8-13. Van Miller et al (1977) exposed male Sprague-Dawley rats to various dietary levels of TCDD ranging from 0.001 ppb (µg/kg) and 1 ppb (yg/kg) to 1 ppm (mg/kg) for 78 weeks. Pronounced mortality was observed at higher dose-rates. 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 the 5 ppb dietary level (yg/kg feed), 4 squamous cell tumours of the lung, A neoplastic nodules (hyperplastic nodules) and 2 cholangiocarcinomas of the liver were found in 7 rats.

Kociba et al (1978) fed groups of 50 male and female Sprague-Dawley rats 0.1, 0.01 and 0.001 yg 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 ppt of TCDD (ng/kg feed) in the 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, the lungs, the hard palate/nasal turbinates and the tongue. In the female rats that had received doses of 0.1 and 0.01 yg/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 yg TCDD/kg body weight a statistically significant increase of hepatocellular carcinomas. Epithelial tumours along the respiratory tract, tongue and hard palate were well differentiated squamous cell carcinomas.

There was an increased incidence of squamous cell carcinomas of the hard palate and nasal turbinate, as compared with the controls, in both male and female rats receiving 0.1 yg 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 that may be secondary to an effect on the hormonal functions of different glands. This decrease was in some instances statistically significant. None of these tumours metastasized.

In a similar study (Kociba et al., 1979) with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) free from TCDD, hexachlorodibenzo-p-dioxin, heptachlorodibenzo-p-dioxin and octachlorodibenzo-p-dioxin, as analyzed by gaschromatography-mass spectrometry with detection Limits of 0.33, 0.12, 0.40 and 0.40 yg/kg for respective compounds, no oncogenic response in rats could be demonstrated.

Two groups of 100 male and 100 female, 10-week old, random-bred Swiss H/Riop mice were given weekly oral doses of 70 mg/kg body weight 2,4,5-trichlorophenoxyethanol (TCPE), together with 0.7 yg TCDD/kg body weight or 0.007 yg TCDD/kg body weight in 0.5% carboxymethyl cellulose by gastric intubation for 12 months. The TCPE used for the 1st group contained 10 mg/kg TCDD and that for the second group 0.1 mg/kg TCDD. The incidence rates of liver tumours in males after 2 years were reported to be 43% and 54% in the two treated groups, compared with 15-20% in the untreated mice of the colony that survived up to 3 years. 3 additional groups of mice were given 7 mg TCPE/kg body weight with 0.0007 yg TCDD/kg body weight, 0.7 mg TCPE/kg body weight 0.00007 yg TCDD/kg body weight, or 7 mg TCPE/kg body weight with 0.7 yg TCDD/kg body weight. The incidence rates of liver tumours in males after 2 years were 15-21% in the 3 groups, compared with 28% in the controls given 0.5% carboxymethyl cellulose alone (Toth et al. 1977). In the follow-up of the same study, Toth et al. (1979) found that in the dose range of 0.07 to 0.0007 yg TCDD per kg diet did not influence tumour frequency, but TCDD had a liver tumour enhancing effect at a higher dose of 0.7 yg per kg diet.

Two recent studies concerning the carcinogenicity of TCDD are also available (NIH, 1982a; NIH, 1982b). TCDD used in these studies was reported to be 99.4% pure based on a 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 8-13. The statistical analysis was performed similar to that in the dermal study. 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.

In the male rats, increased incidence of follicular-cell adenomas in the thyroid were dose-related and were significantly higher (P 6 0.001) in the high-dose group than in the vehicle controls (1/69, 1%; 5/48, 10%; 6/50, 12%; 10/50, 20% for vehicle controls, the low, medium and high doses, respectively). Similar in the female rats, an increase (though not statistically significant) was seen in the high-dose group (3/73, 4%; 2/45, 4%; 1/49, 2%; 6/47, 13%). The incidence of neoplastic nodules of the liver in the high-dose group of female mice was significantly (P 6 0.006) higher than that in the vehicle-control group (5/75, 7%; 1/49, 2%; 3/50, 6%; 12/49, 24%).

In male and female mice, incidences of hepatocellular carcinomas were dose-related and the incidences in the high-dose groups were significantly (P 6 0.002 and 0.014, respectively) higher than those in the corresponding vehicle-control groups (males; 8/73, 11%; 9/49, 18%; 8/49, 16%; 17/50, 34%; females: 1/73, 1%; 2/50, 4%; 2/48, 4%; 6/47, 13%).

Follicular-cell adenomas in the thyroid occurred at dose-related incidences in female mice, and were significantly (P 6 0.009) higher in the high-dose groups than those in the vehicle-controls (0/69, 0%; 3/50, 6%; 1/47, 2%; 5/46, 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.

In the dermal study (NIH, 1982b) male and female Swiss-Webster mice were about 6 weeks old in 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 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 DMBA was significantly higher than that of the controls (P 6 0.007 and *P* 6 0.010, respectively). An increase in the same tumour type, although not statistically significant (P= 0.084), was also observed in the male mice (3/42, 7%; 6/28, 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. The study has been, however, criticized for some fails including that a maximal tolerated dose (MTD) was not determined, especially in male mice, only one dose per sex was used, and the number of mice (30) in the TCDD exposed groups was considered marginal.

A 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins (HxCDDs) has been tested for carcinogenicity in rats and mice treated by gavage and by dermal application to mice (NIH, 1980 a and b). The following impurities were detected in the mixture: PeCDD 0.04%, TCDD 0.09 ± 0.03%, TriCDD 0.004% and BromoPeCDD < 0.004%. The specific isomers of these impurities were not identified. Dosages and duration for the gavage studies (NIH 1981a) are given in table 8-13.

In both species and either sex only tumors of the liver occurred at a significantly greater incidence than controls. In male rats and male and female mice, the liver tumor incidence was significantly increased over control values only in the high dose groups (5 µg/kg/week), while in female rats the incidence was significantly greater at both the medium- and high-dose levels (2.5-5 µg/kg/week).

In the dermal study, no treatment-related tumors were recorded in either the carcinogenicity bioassay or the tumor promotion assay using DMBA as an initiator (NIH 1980b). It was concluded that the tested mixture of HxCDDs was carcinogenic to rats and mice following administration by gavage. However, there was no tumorigenic activity when HxCDD was applied to mouse skin.

2,7-dichlorodibenzo-p-dioxin and dibenzo-p-dioxin were found not to be carcinogenic in chronic feeding studies on mice and rats of either sex (NCI 1977, NCI 1979).

In a Limited study (table 8-13) on field exposed beach mice (Peramyscus polienotus) the only statistically significant finding was an increase in liver to body weight ratios. The mice were exposed from soil in an area heavily treated with 2,3,7,8-TCDD-contaminated 2,4,5-T. The authors estimated the daily 2,3,7,8-TCDD-dose to 0.0012 µg/kg body weight from the levels analyzed in the livers of the animals. It was noted that this exposure was much lower than the exposures used in laboratory studies to produce tumors.

When the International Agency for Research on Cancer (IARC) evaluated the experimental animal data for TCDD in 1977, the more recent information summarized in this chapter was not available to the IARC working group. In February 1978, the IARC convened, jointly with the National Institute of Environmental Health Sciences (NIEHS) of the USA, a meeting on the long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. The working group concluded that the two reports (Kociba et al 1978; Van Miller et al 1977) demonstrated that chronic administration of 2,3,7,8-TCDD caused increased incidence of neoplasms, but did not show whether 2,3,7,8-TCDD acted as an initiator or promoter.
The IARC/NIES working group noted that at least 24 long-term carcinogenicity studies using rats and mice were in progress in 1978. The results from many of these studies are still not yet available.

8.7.2 Short term and interaction studies

Poland & Glover (1979) estimated the maximum in vivo covalent binding of TCDD to rat liver protein, ribosomal RNA, and DNA. ^{3}H-TCDD (39 Ci/mmol) was administered to immature male and female Sprague-Dawley rats (105-135 g) as a single i.p. injection of 7.5 µg/kg. The rats were killed 12 hr, 24 hr, 48 hr or 7 days after dosing with TCDD, and the concentration of radioactivity in the liver varied from 18 to 64% of the administered dose. Only a small fraction was associated with the purified macromolecular fractions. The radioactivity associated with rRNA and DNA was very low and essentially all of unextracted radioactivity was associated with protein (0.03 to 0.1% of the total radioactivity in the liver). The estimated maximum amount of ^{3}H-TCDD that could have been covalently bound to DNA was 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 admi-

nistration of TCDD did not significantly alter the amount of unextractable ^{3}H-TCDD associated with any macromolecular fraction. Similarly, there was no difference in the levels of ^{3}H-TCDD associated with protein, rRNA, or DNA in male or female rats pretreated with TCDD.

Several groups of investigators have found TCDD to be a potent 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 cr nucleotide residue. The maximum binding of TCDD is 4 to 6 orders of magnitude lower than that of most chemical carcinogens, and of questionable biological significance. The results obtained in the study of Poland and Glover (1979) thus indicate it is unlikely that the mechanism of TCDD-induced carcinogenesis would include any covalent binding of TCDD.

In dermal carcinogenesis studies 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 (Di Giovanni et al 1977). In these studies 12-0-tetracanyl-phorbol-13-acetate (TPA) was used as a promoter. When TCDD and 7,13-dimethylbenzanthracene were given together, a slight additive effect was found.

As mentioned earlier in a study on a HxCDD-mixture, no treatment-related tumors were found in a tumor promotion test on mice using DMBA as an initiator (NIH 1980b).

Evaluation of the possible role of TCDD as a promotor in diethylnitrosamine-induced hepatocarcinogenesis was made 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 hr after a 70% hepatectomy, and TCDD treatment (0.14 or 1.4 yg/kg s.c. once every 2 weeks for 7 months) was started one week after the hepatectomy. The promoting effect of TCDD on 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 carsinomas, were greatly increased in number, total volume, and phenotypic heterogeneity by the administration of TCDD. A significant incidence of hepatocellular carcinomas (5 of 7) was observed in the DEN-treated rats which were given the high dose of TCDD, but no carcinomas were seen in the rats treated with DEN only (0 of 4). The

results indicated that TCDO was a potent promoting agent for hepatocarcinogenesis, and according to authors it seems a reasonable hypothesis that all the tumors associated with the chronic administration of TCOD arise from its promoting activity of cells already "initiated" by exposure to the environment.

Studies in a two-stage system of mouse skin tumorigenesis (Berry et al., 1979), which allows one to evaluate the initiation and promotion phases of carcinogenesis individually, have demonstrated that TCDD do not promote the development of skin tumours at a dose of 0.1 yg twice weekly. In the same study when the animals were pretreated with 1.0 yg TCDD for 1,3 or 5 days prior to initiation with (DMBA) 7,12-dimethylbenz(a)-anthracene, TCDD was shown to act as a potent inhibitor of PAH-induced skin tumour initiation. Almost complete inhibition (98%) is achieved with a single nontoxic topical dose of 0.1 yg, and 3 days pretreatment with 0.01 yg TCDD, gave already over 80 per cents' inhibition. The authors suggest 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 induction by other chemicals by enhancing the metabolism of these xenobiotics.

Poland et al (1982) studied the promoting effects of 2,3,7,8-TCDD in the mouse skin two-stage tumorogenesis model and compared the effects of 2,3,7,8-TCDD and TPA in DMBA-initiated HRS/S mice which were either heterozygous or homozygous for the recessive "hairless" trait.
2,3,7,8-TCDD was found to have a tumorpromoting effect only in the homozygous mice. The data also suggested to the authors that 2,3,7,8-TCDD might act promoting by a mechanism different from TPA.

The interaction of 2,3,7,8-TCDD with 3-methyl-cholanthene (3-MC) was studied by Kuori et al (1978) who found that 2,3,7,8-TCDD was a cocarcinogen with 3-MC when administered by subcutaneous injection.

The anticarcinogenic effect of 2,3,7,8-TCDD has been reported by Cohen et al. (1979) and by DiGiovanni et al. (1979). When 2,3,7,8-TCDD was topically applied to Sencar or CD-1 mice 72 hours prior to the administration of either 7,12-dimethylbenz(a)anthracene (DMBA) (10 nmol) or benzo-(a)pyrene (B(a)P)

(100 nmol), it markedly decreased the skin tumor initiation by both DMBA and B(a)P. 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 B(a)P to DNA. However, the hydrocarbon-deoxyribonucleoside adducts from DNA of TCDD-pretreated mice showed a striking absence of B(a)P-7,8-dihyrodiol-9,10-epoxide adduct bound to guanine. It is suggested, accordingly, that the formation of this adduct may be a critical step in B(a)P-induced mouse skin carcinogenesis. In further studies of the tumor inhibitory effect of 2,3,7,8-TCDD (Di Giovanni et al 1980), it was demonstrated that exposure of CD-1 mice to 2,3,7,8-TCDD 3 days before initiation with BaP or 3-MC resulted in a decreaed tumor yield as compared to acetone-pretreated animals while treatment with 2,3,7,8-TCDD 5 minutes before and 1 day after initiation failed to affect the tumor yield. However, when 2,3,7,8-TCDD was administered 3 days or 5 minutes before or 1 day after initiation with BaP-diol epoxide, there was a decreased tumor yield in all cases. The authors concluded that the ability cf 2,3,7,8-TCDD to inhibit tumor yield, when administered after the BaP-diol epoxide, indicated the possible existence of more than one mechanism behind the anticarcinogenic effect of 2,3,7,8-TCDD.

8.8 Mechanism of toxicity

Despite massive research effort attempted at elucidating the ultimate event behind the toxic action of TCDD no such information is yet available.
The toxicity cf TCDD apparently depends or, the fact that the four lateral positions of the molecule are occupied by chlorine. Toxicity decreases with decreasing lateral substituents and increasing chlorine substitution. TCDD poisoning involves many different types of toxic symtoms and these symtoms vary from species to species and from tissue to tissue both quantitatively and qualitatively. Furthermore, age and sex related differences in sensitivity has been reported even at very high doses. Characteristic for TCDD poisoning is also the delay in toxicity, from 2 weeks to 2 months, seen in all species. Although the metabolism and toxicokinetics of TCDD varies between species these differences are not enough to explain the variabilities in sensitivity to TCDD toxicity.
Existing data suggest that the initial event of TCDD poisoning is the binding of TCDD to a receptor. This complex, wether of cytosolic or nuclear origin, excerts its action in the nucleus by triggering a pleiotropic response

including induction of mixed function oxidases. The present knowledge, however, rule out enzyme induction per se as being the cause of toxicity and death.
Recent research indicate an involvement of TCDD in processes regulating cellular differentiation and/or division. Alterations in the regulation of such processes that are present, though not equally active, in all cells throughout the organism would be expected to result in effects that varies among tissues as well as among species.

8.8.1 Receptor mediated toxicity

The necessary first step in induction of cytochrome P-448 synthesis and of AHH activity due to TCDO is the specific binding of the inducer to a cytosolic receptor protein (Okey et al 1979, Poland and Glover 1979). The hepatic receptor protein has a Stokes radius of 6.6 nm, a sedimentation coefficient of 5.0 S and a molecular weight of 136 000. The TCDD-receptor complex, but not the receptor alone, do bind to isolated DNA. The receptor protein has been found also in extrahepatic tissues (Carlstedt-Duke 1979, Carlstedt-Duke et al 1979, 1981, Mason and Okey 1982). The TCDD-receptor complex is proposed to translocate into the nucleus where binding to DNA activate a number of structural genes to initiate transcriptional processes (Greenlee and Poland 1979, Mason and Okey 1982, Okey et al 1979, 1980, Poellinger et al 1982, Poland and Knutson 1982, Tukey et al 1982). Recently Whitlock and Galeazzi (1984) reported that binding of TCDD to the receptor is a nuclear rather than a cytosolic event. There are similarities in the biochemical behaviour between the TCDD-receptor and steroid hormone receptors. Thus it has been postulated that there is a natural ligand, yet not identified, possibly of endogenous but obviously not of steroid origin (Neal et al 1979, Poland and Glover 1976).
The most convincing evidence for the importance of the receptor in TCDD induced toxicity are based on structure-activity relationships. The binding affinities of TCDD and analogous compounds to the receptor have been demonstrated to correlate well with their biological potencies, especially with the ability to induce hepatic AHH activity but also to lethal potencies (tables 8-14 and 8-15) (Bandiera et al 1984, Knutson and Poland 1982, Poland et al 1976, Poland and Kende 1977). The observation that the administration of a dose of 2,3,7,8-TCDF, 10 µg/kg body weight inactive per se, simultaneously

with an active dose of TCDD, 1.2 µg/kg body weight, to C57Bl/6 mice reduced the immunosuppressive and enzyme inducing capacity of TCDD suggests a competitive effect on the receptor Level (Rizzardini et al 1983).

Polymorphism in the Ah-locus, sugpested as the 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-Methylcholanthrene (3-MC) and (c) sensitivity to the ulcerative action of 7,12-dimethylbenzanthracene on the skin. Ah-nonresponsive mice, e.q. DBA/2, lack these attributes (Nebert et al 1982). 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-nonresponsive strains of mice. However, in each case it appears that the dose required to produce the effect in an Ah-nonresponsive strain is approximately 10-fold greater than that needed for a responsive strain. By using crosses and backcrosses of C57BL/6 and DBA/2 mice it has been shown that sensitivity to TCDD-induced thymic atrophy and immunesystem disturbances, skinlesions of hairless mice and teratogenic effects (see 8.5?) all segregate with the Ah-locus. Furthermore data from studies of DBA/2 mice given either single or multiple doses of TCDD (Jones and Sweeney 1980, Smith et al 1981) also 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 (Jones and Greig 1975, Smith el al 1981, Vo? et al 1974). TCDD-induced hepatic porphyria has also been shown to segregate with the Ah-locus in mice (Jones and Sweeney 1980). However, recently Greig et al (1984) found that additional genetic loci must be involved in this leison. Recent findings suggest that the correlative differences between the C57BL/6J and DBA/2 strains of mice, in terms of altered specific binding of TCDD and sensitivity to this compound, may be unique and not necessarily applicable to other species (Gasiewicz and Rucci 1984).

Less convincing data for the model of a receptor mediated toxicity of TCDD arise from studies of toxicity, receptor levels and/or AHH-induction of TCDD in various species, tissues and cell cultures. Despite enormous variability in recorded LD_{50}-values for guinea pig, rat, mouse, rabbit and hamster (table 8-1) are the amount and physical properties of the hepatic as well as extrahepatic receptors comparable in these species (Gasiewicz and Rucci 1984,

Poland and Knutson 1982). Furthermore, although recorded LD_{50}-values for TCDD vary more than 100 times in chick embryo, C3H/HeN mice and Sprague-Dawley rat, the ED_{50}-doses for AHH induction in these species are comparable (Poland and Glover 197A). In the guinea pig, the most TCDD-susceptible species, AHH-induction is not a prominent sign even at lethal doses. A number of cell types, including primary cultures, 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 (Knutson and Poland 1980).

Available data thus suggest that the receptor for TCDD is not sufficient though it seems to be a pre-requisite, for the mediation of toxicity.

8.8.2 Impairment of normal cellular regulatory systems

Taken together the diverse pattern of toxic effects, the species- and tissue-specific responses, the time-course for effects to occur as well as the non-toxic action of TCDD on most cell cultures in vitro seem to indicate that TCDD-toxicity occurs as an impairment of a normal cellular regulatory system. Such a system ought to be present in all cells throughout the organism though the activity may vary with celltype, tissue, age, sex and species.

8.8.2.1 Endocrine imbalance

In many aspects symtoms of TCDD poisoning mimic endocrine imbalance, however, no evidence exist to support the direct involvment of steroid hormones in the toxic action of TCDD.

8.8.2.2 Body weight regulation

The most reliable and consistent symtom of TCDD poisoning among all experimental animals is weight loss. The cause of the body weight loss seems to be reduced food intake apparently occuring secondary to a physiological adjustment to reduce the body weight to a maintenance level lower than normal. The physiological trigger for this body weight set-point might be a target for TCDD.

8.8.2.3 Plasmamembrane function

The most intriguing aspect of the changes in the surface characteristics of the plasmamembranes induced by TCDD in vivo (see 8.4.1.2) is the resemblance with changes occuring in precancerous and transformed cells (Hynes 1979, Pitot and Sirica 1980). Such changes, including reduction of gap junctions and surface glycoproteins, are expected to curtail cell-cell communication and to reduce intercellular recognition and attachment events implicated in the process of tumor 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, of which the 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. TCDD:s lack of effect in vitro would imply that the promoter effect is mediated through some in vivo process and not by its direct interaction on plasmamembranes.

8.8.2.4 Impaired vitamin A storage

Many of the symtoms of TCDD poisoning, including failure of normal growth, keratosis, epithelial leisons, immunosuppression, reproductive and teratological effects are similar to the effects of dietary vitamin A deficiency (Thunberg et al 1980). The most intriguing similarities between symtoms due to vitamin A deficiency or TCDD-toxicity concerns epithelial tissues and especially the effects on the process of keratinization. TCDD induces terminal differentiation of epithelial tissues both in vivo and in vitro.
However, lack of epithelial degeneration (programmed celldeath) 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 the 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 a 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 the nature of keratins synthesized by controlling the content of the corresponding mRNA (Fuchs and Green 1981). Keratins constitute a cytoskeleton in epithelial cells and the keratin pattern has been suggested to be a marker for epithelial differentiation (Sun et al 1979, 1983). 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 shorter keratins. This pattern was reversed by the addition of vitamin A to the medium. Each tissue and cell type did adjust its own synthesis of keratins differently depending on the vitamin A concentration in the medium (Fuchs and Green 1981). The ability of TCDD to impair vitamin A storage may be responsible for some of the toxic effects produced by TCDD. However, as dietary vitamin A seems unable to counteract the toxic effect of TCDD it is possible that the effect on vitamin A storage is secondary to TCDD-toxicity.

8.8.3 Lipid peroxidation

Based on indirect lines of evidence Sweeney and Jones (1983) proposed that increased *in vivo* lipid peroxidation, resulting in the formation of free radicals, might be a possible mechanism of TCDD-toxicity. First, lipofuscin pigments, considered to be byproducts of lipid peroxidation, accumulate in the heart of TCDD-treated rats (Albro et al 1978). Secondly, iron deficiency inhibits *in vitro* lipid peroxidation (Bus and Gibson 1979, Sweeney et al 1979) and has been demonstrated to reduce hepatic toxicity *in vivo* in TCDD-treated rats (Sweeney et al 1979). Thirdly, 0.25% butylated hydroxyanisol in the diet provided protection from TCDD-induced porphyria and lipid accumulation in mice. On the contrary 0.01% vitamin E, an other anti-oxidant, in the diet had no protective effect. Recently, Stohs et al (1983) demonstrated increased *in vivo* and *in vitro* lipid peroxidation in female SD rats administered 3 daily doses of 10, 20 and 40 µg TCDD/kg body weight or a single 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.

9. EFFECT ON MAN - EPIDEMIOLOGICAL AND CASE STUDIES

9.1 Occupational studies

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 first described by Herzheimer (1899). In addition to the halogenated phenols, chloracne has been caused by a number of chlorinated compounds such as the chlorinated biphenyls and chlorinated naphthalenes (Crow 1970; Kimbrough 1974). 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. Comedons 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; in particular, the malar areas, the jaws and the regions behind the ears are affected. 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, the neck,

back, abdomen, and the outer thighs and genitalia may also be involved (Crow 1970). 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 United States, 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 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 report followed. The technique of obtaining chlorine gas consisted of an electrolytic procedure where a mixture of potassium-, sodium and magnesium chloride 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 electrolytic procedure and cleaned the reaction vessels, where those afflicted. Already at this time chlorinated phenolic compounds where thought of as possible noxious agents (Fraenkel 1902). This however could never be proven and at present when satisfactory analytical techniques are available no analysis of the so called "tuffy tar" has been carried out.

The skin damage caused by chlorinated organic compounds entered a second stage during the great war 1914-1918. At this time perchlorinated naphtalenes had come into use as isolation material e.g. in radio and electronic industry. The first description of Pernakrankheit is that by Wauer 1918. The use of the unspecified technical mixture of chlorinated naphtalenes spred all over the world and caused numerous intoxications notably among workers in manufacture. The perna disease has been summarized by von Wedel et al 19A3 and particularily 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 experimental animals and man serious liver damage occurred consisting of livernecrosis (toxic jaundice, acute yellow liver atrophy). Among several hundred cases with chloracne due to these compounds Bauer tabulated 24 deaths due to toxic jaundice and 14 recoveries. I should be pointed out that a fulminant liver disease with jaundice of this kind is an extremely rare condition, by comparison it has never occurred by exposure to TCP and TCOD

described below. Note should also be taken of the fact that not only were the perchlorated naphtalenes an ill identified mixture of chemical individuals of this group but exposure frequently occurred at the same time also to mixtures of chlorinated biphenyls, the latter now known to be contaminated with chlorinated dibenzofuranes. The potentiation of toxicity by these mixtures and other chlorinated compounds are discussed by Drinker et al 1937, Greenburg et al 1939, won Wedel et al 1943 and Risse-Sunderman 1959.

Accidents in chemical plants involved in the manufacture of chlorinated phenolic compounds have been tabulated (Table 9.1). I should be stressed that all these intoxications are due to mixtures e.g. TCP + TCDD and other compounds. Summaries of the industrial accidents are to be found in Holmstedt 1980 and Hay 1982 and only some of them will be dealt with here.

The first intoxication with a mixture containing TCDD, although the chemical structure was not given, occurred on the 5th of February 1910. Five people are 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, thesis 1914). Except for the at that time wellknown dermatological lesions two persons are reported to have suffered from "muscle rheumatism" during hospitalisation and one from infection in the airways but otherwise no signs and symptoms of general disease. It has been said that TCDD could have been discovered already then but instead this dermatological thesis gathered dust in a university library (Dohmeier and Janson 1983). Whale, however, in his thesis emphazised that this intoxication was not due to any of the by then well known chlorinated naphthalene derivatives.

The second reported cases of industrial poisoning, due to the formation of TCDD in uncontrolled exothermic reactions occurring during the manufacture of TCP, were seen in 1949 at a 2,4,5-T-producing factory in Nitro, West Virginia, USA. The temperature in one of the reactors containing tetrachlorobenzene, methanol and sodium hydroxide arose, a relief valve opened and the contents of the vessel was discharged into the interior of the building and over a wide area outside of the building. Two hundered twentyeight persons were affected.

Symptoms included chloracne, nausea, vomiting, headaches, severe muscular aches and pains, fatigue, emotional instability and intolerance to cold. Laboratory findings showed raised total 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 employee husbands. A man from the nearby town who purchased a truck which 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 fatiguabilty, the manifestations of peripheral neuropathy. Liver tests were negative 4 years later. Mild residue of acne were, however, common. TCDD was still unknown. The follow-up of this accident will be discussed under 9.3.

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 4 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 came down with chloracne (Hofmann 1957; Goldmann 1972). In 1968 and 1969 the building containing the autoclaves was dismantled. Goldmann (1972, 1973) conducted a study of this incident in 42 workers. In 21 cases, the chloracne was preceded by a non-specific dermatitis and in 2 cases very persistent chronic conjunctivitis and blepharitis were observed; 14 cases also showed involvement of other organs. In 4 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 noted. In 5 instances the upper respiratory tract was involved with tracheitis and bronchitis, there was 1 instance of haemorrhagic pleuritis and 1 instance of afebrile gingivitis and stomatitis. In a number of cases a high susceptibility to infection was noted, sometimes accompanied by a decrease in gammaglobulin; 1 worker died of pancreatitis, in 7 cases the central nervous system was affected, 3 instances of toxic polyneuritis were recorded, and in 2 instances hearing, sense of smell and taste were impaired. Subjective symptoms similar to those described by Bauer et al

(1961) were also noted. The child of one of these workers also developed chloracne, and in most of the workers active chloracne persisted for many years - in 1 instance for 18 years. Follow-up studies see 9.3.

Of particular interest is the much overlooked thesis 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 also 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 hypesthesisas, as well as extreme irritability. One patient became psychotic and committed suicide. In addition, some of the patients complained of impotence.

10 workers at this chemical factory were followed for five years by Risse-Sundermann, who gives detailed case histories. In addition to the above mentioned signs and symptoms she noticed swollen lymph glands and 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 preceeded that of the skin manifestations (e.g. tiredness, depression, lack of appetite, stomach pains, sexual dysfunction).

In 1961 Bauer et al reported a study of workers affected by 3 different outbreaks of chloracne. In this study more than 100 workers were examined. The 31 Hamburg workers had been exposed 5 years ealier. 9 of them were examined in detail and their symptoms tabulated. The illness progressed as follows: 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 irritation 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 that did not stain positive for iron. A virushepatitis 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. Follow-up studies are reported under 9.3.

In Northern Italy following the malfunction of a reaction vessel in which 2,4,5-trichlorophenol was produced, the temperature in the vessel increased and an intense black vapour filled the work-room covering everything with a black deposit; 5 workers engaged in clean-up operations developed chloracne (Hofmann & Meneghini 1962).

> "Case No. 1: G.E., age 15, worker in a chemical plant employed in preparing trichlorophenol from tetrachlorobenzene. Following a breakdown in the boiler used for preparing the final product in the department where the patient worked, a rise in the temperature caused the development of intense blackish vapors from the active substances; they were deposited on all of the surfaces in the environment as a tarry blanket. The patient then cleaned the department and the boilers. A few days after the cleaning period began, the subject observed many blackish blackheads, folliculitis and superficial nodular elements on the face, forearms, and neck. A slow but progressive generalization of the dermatosis to the trunk, scalp, and lower extremities (to a lesser degree) then followed. Several months after the onset of the first symptoms, the patients was hospitalized in this clinic. There were no data of particular importance in the remote history. He had had a mild seborrheic condition since puberty. The following was found on general objective examination: normal type in good general physical and mental condition. No particular organic changes. Current laboratory tests revealed no damage to the renal and hepatic parenchyma."

With regard to systemic effects the authors state:

"None of our patients exhibited any involvement of the general or visceral condition (even 16 months after the final exposure to the noxa) which could be related to exposure to the causal agent - in contrast with the reports of several authors".

The mixture in the reactor was applied to the skin of both volounteers and exposed workers and subsequent biopsy showed the characteristic picture chloracne. One of the employees (case 1 GE) was interviewed in 1980 and still had outburst of chloracne but no other complaints (Holmstedt 1980).

Duverne et al (1964) reported a case that occurred at a plant at Lyon, France, where products that used 2,4,5-trichlorophenol as a starting material were manufactured. This worker developed chloracne as well as serofibrinous pleuritis. Ten workers at a plant near Grenoble, France, also developed chloracne. They produced 2,4,5-trichlorophenol that served as the starting mterial for phenoxy pesticides and germicides for cosmetics. These workers also 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 tetrachloro-benzene 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 at the Chesterfield plant, 2 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) also cites two incidents that were reported in the Quarterly Safety Summary of the Association of British Chemical Manufacturers, 1970, 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. Follow-up studies see 9.3.

According to Dalderup (1974), an explosion occurred at a 2,4,5-T factory in the Netherlands in the early 1960s. The plant was demolished and buried at sea in May and June 1973. The extensive safety measures taken during the demolition process are described (see also Holmstedt 1980). Many unclear points exist concerning this episode the description of which is incomplete.

In the USA, one outbreak of chloracne occurred among workers manufacturing 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (Bleiberg et al 1964) 29 workers had chloracne and 11 of these had elevated urinary uroporphyrins and exhibited varying degrees of acquired porphyria cutanea tarda. At least 1 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. Moderate to severe chloracne was present in 13 (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 i 7.6 years (mean ± 1 SD). Twenty-six of the workers seen by Bleiberg et al (1964) were also seen in the follow-up study (Dr Alan Poland, personal communication). The trichlorophenol manufactured in this plant contained 10-25 ppm (mg/kg) TCDD. 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 ppm (mg/kg) TCDD. The cases of porfyria will be discussed under 9.3.

Additional occupational exposure to TCDD occurred in 1964 at the Dow Chemical Company. In 1964 the Dow Chemical Company (President's Science Advisory Committee 1971) changed the reaction conditions for their 2,4,5-T production. Subsequently chloracne developed in 60 workers. Follow-up studies will be found under 9.3.

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, the now famous accident occurred at the ICMESA plant at Meda, near Seveso, Italy, 12 workers were present; 3 or 4 weeks after the accident all 176 workers of the plant were examined. Chloracne was suspected in 1 of them; the others showed minor symptoms that could not be correlated with exposure. Alkaline phosphatase and gamma-glutamyltransferase seemed slightly increased in 32 and 37 cases respectively, 5 workers showed a reduction in their -amino-levulinate dehydratase blood levels, and 3 moderately increased urinary -aminolevulinic acid (Zedda et al 1976). Similar findings were reported by Fara (1976) and Reggiani (1978). The follow-up of the general population will be described under 9.2.

9.2 General population studies

A high level of localized environmental exposure recorded in the literature (section 4.3) occurred in a small area of Missouri (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 to 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, 2 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 0.56-0.65% trichlorphenol, 31.8-33.0 ppm (mg/kg) TCDD, and 1350-1590 ppm (mg/kg) polychlorinated biphenyls. Because of this finding, the episode was reinvestigated. It was found that the salvage oil company that sprayed the 3 arenas routinely collected discarded motor oil and lubricants from over 2 000 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 the TCDD source. This company had manufactured trichlorophenol as an intermediate for hexachlorophene. The production of 2,4,5-trichlorophenol 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. He picked up 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. The residue storage tank which contained the chemical waste material had remained undisturbed since 1971. A sample collected in 1974 revealed a TCDD concentration of 300-356 ppm (mg/kg). Efforts by local and Federal governmen officials to dispose of the material in the residue tank have thus far been unsuccessful. Soil samples from arenas where contaminated dirt had been dumped in 1974 contained trichlorophenol levels that ranged from 1.5-32.6 ppm (mg/kg), TCDD levels that ranged from 0.22-0.85 ppm (mg/kg), and polychlorinated biphenyl levels that ranged from 10-25 ppm (mg/kg). The TCDD levels in dirt collected from the same dump sites in 1976 were within the same range (Liddle et al 1977).

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 individuals 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. The signs and symptoms observed in these individuals are listed in Table 9-2. Evaluation of the 3 female patients 5.4 years after exposure to TCDD showed them to be in good health (Beale et al 1977).

The scientific follow-up on the Seveso population was guided an international steering group headed by professor M A Klingberg. The group ended 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 Lombarchia 1984) a total of 193 persons had displayed symptoms of chloracne but in the beginning of 1984 only 20 presented active symptoms. 15-20 days exposure to TCDD soil levels of 270-1200 $\mu g/m^2$ clearly caused an enhanced incidence of chloracne. No . . . . . of disturbance of biochemical functions were seen when the exposure had been limited to soil with TCDD-levels at or below 30-70 $\mu g/m^2$.

The group also concluded that some earlier reported findings such as a decrease in motor nerve conduction velocity in some individuals had not been confirmed in later evaluations.

Two other findings have been reported, A significant increase in urinary glucaric acid levels, indicating an increased microsomal enzyme activity, was found in exposed children 3 years after exposure (n = 67) as compared to non exposed (n = 86) (Ideo et al 1982).

The steering group found the data difficult to evaluate as analytical and individual biological variabilities were not explained (Regione Lombardia 1982).

Studies performed on rate of spontaneous abortions and birth defects in the Seveso area do not allow any conclusions to be drawn (Tognoni and Bonaccorsi 1982). Fortunately there is no history of a pregnant woman having chloracne.

The hypothesis that low exposure might cause pre-pregnancy or pregnancy effects that adversely affect the outlcome was tested using several exposure models. The only finding was a slightly higher rate of hemangioma among newborns in the exposed group. However, this showed up only with one of the exposure models. It is doubtful whether this can be due to TCDD exposure (Regione Lombardia 1984).
Several epidemiological follow-up studies are ongoing in the area.

From 1960 to 1969 a mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (Agent Orange) (section 4.2) which was contaminated with TCDD, concentration ranging from 0.5 to 47 ppm (Kearny et al 1973), was sprayed over arenas of Vietnam 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. In 1970, Cutting et al conducted a study to determine whether the inadvertent exposure of the general population in Vietnam to this herbicide could have resulted in an increase in birth defects. The periods 1960-1965 and 1966-1969 were compared, i.e. a period of minimal spraying with a period of heavy spraying. The number of stillbirths in the different parts of Vietnam varied a great deal and a fluctuation in the incidence of malformations was also noted which may have been a reflection of the reporting system.

For instance, the stillbirth rate at the Long Dien Hospital fluctated from 1.4 in 1962 to 13.4/1000 live births in 1965. In 1966-1969, the Qui Nhon Provincial Hospital had a much higher stillbirth rate that ranged from 51 to 75/1000 live births. Unfortunately, no information is available from this hospital for the earlier time period. Data for the period 1960-1965 were collected from 8 hospitals (Long Dien Hospital, Baria Provincial Hospital, Da Lat Procinvial Hospital, Tu-Du Maternity Hospital, Saigon, Hung Vuong Maternity Hospital, Saigon, Bien Hoa Provincial Hospital, Tan An Provincial Hospital). The 2 hospitals in Saigon reported about 59% of the live births together with a total of 9 477 stillbirths and 1 802 malformations. Incomplete data collected from 15 additional hospitals for the period 1966-1969 report a total of 99 906 live births and a stillbirth rate ranging from 4.7 to 62/1000 live births.

According to Wilson (1971), the study by Cutting et al was fraught with difficulties. The estimated population of South Vietnam in 1970 was 18 million with a birth rate o 35-42/1000. The yield in 1970 should therefore have been 630-750 000 births. However, the total number of births recorded for the period 1960-1969 was only 480 087, and the births included in the study by Cutting et al (1970) were unevenly distributed over the country, the 3 hospitals in the capital area accounting for over 67%. The population that had been most heavily exposed to 2,4,5-T came from relatively remote and sparsely populated areas and probably did not exceed 5% of the total population of Vietnam, and a significant proportion of this exposed population segment consisted of "montagnards" who usually did not deliver in hospitals. All this in addition to inadequate records, particularly incomplete recording of congenital malformations, makes it impossible to resolve these problems and to conduct any meaningful statistical comparisons. These deficiencies have also been pointed out by Meselson et al (1972).

Tung (1973) reported an increased incidence of liver tumours in Vietnam. 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. Tungs work has been chritisized by Hay 1982.

> "Tung has said that the use of the herbicide Agent Orange by United States military forces in Vietnam may be associated with the increase in primary liver cancer in the country. Controlled epidemiological surveys to support this contention have not been conducted. The latency period

between exposure to the herbicides and the noted increase in Liver cancer is less than ten years. As most known carcinogens have a Latency period of 20-30 years, the Link between TCDO exposure and liver cancer in Vietnam is thought by many scientists to be slim."

A continuation of tungs work is that by Van (1983, 1984). This work has been commented upon by Hay (1984):

"Some in vitro tests suggest that dioxin, a contaminant associated with the phenoxy herbicide 2,4,5-T, is a mutagen and thus perhaps as well a mammalian (including human) carcinogen. Direct tests for dioxin carcinogenicity using rodents have confirmed that the chemical does cause cancer in animals, but whether it does so in humans has not as yet been established.

Disturbing indications exist from Viet Nam that exposure to phenoxy herbicides will cause chromosome aberrations. Supporting evidence for these reports is not available, however, from mammalian laboratory studies or from epidemiological surveys of occupationally exposed individuals in other countries. Careful follow-up studies are recommended."

Dwyer and Epstein (1984) noted the following:

"Van (__) explored the possibility of wartime herbicide exposure in 21 recent cases of liver cancer (primary hepatic carcinoma). Of this group, 6 turned out to have been thus exposed during the Second Indochine War, a value considered to have been high enough (in comparison with a control group of 42) to suggest a cause-effect relationship. It should be mentioned that Van's investigation had been prompted by the finding of Tung (1973) that the proportional incidence of primary hepatic cancers had risen during the war years. However, it must be noted that Van's sample was small and his sampling procedure perhaps biased; and it is also not clear whether his procedures would have been able to discriminate between phenoxy herbicides and other possible confounding factors in the aetiology of the observed tumours (e.g., certain parasites, hepatitis-B virus, aflatoxin). Pham Hoàng Phiet (Cho Ray Hospital, Ho Chi Minh City, unpublished) recently carried out a similar study on 26 cases of primary hepatic cancer (and *52* controls), but in the view of the present authors with inconclusive results. – Finally, it

would be incorrect to statue that the Vietnamese pilot studies reviewed here have provided definitive evidence for the health effects of phenoxy herbicides."

Exposure to TCDD through contact with 2,4,5-T-based herbicides is considered to be of a lower order of magnitude than that experienced by workers exposed to the dioxin in industrial accidents.

In 1979 the United States Air Force (USAF) made the commitment to Congress and to the White House to conduct an epidemiologic study of the possible health effects from chemical exposure in Air Force personnel who conducted aerial herbicide dissemination missions in Vietnam (Operation Ranch Hand) (Lathrop et al, 1984). The purpose of this epidemiologic investigation was to determine whether long-term health effects exist and can be attributed to occupational exposure to herbicides. This study uses a matched cohort design in a non-concurrent prospective setting, incorporating mortality, morbidity, and follow-up studies. The report presents 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.

This baseline report concludes that there is insufficient evidence to support a cause and effect relationship between herbicide exposure and adverse health in the Rach Hand group at this time. The study disclosed numerous medical findings, mostly of a minor or undetermined nature, that require detailed follow-up. In full context, the baseline study results should be viewed as reassuring to the Ranch Handers and to their families at this time.

In Australia, 1980, the Commonwealth Institute of Health agreed to conduct a series of scientific investigations into the health of Vietnam veterans and their families. A special independent unit known as the Australian Veterans Herbicide Studies, now the Australian Veterans Health Studies (AVHS), was set up within the Institute to conduct the studies. After consideration of 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 Vietnam service. This report entitled "Case-control study of congenital animalies and

Vietnam service (Birth defects study) - Report to the minister for veterans' affairs, January 1983. The report is largely negative and so is that of Erikson et al 1984, which studies American veterans in a similar way.

9.3 Long-term effects and epidemiology

Signs and symptoms that have been reported related to accidental exposure to TCOP will be found in table 9.3. However, it should be observed that all the accidents and occupational contamination concern exposure to a mixture of compounds where TCDD is only one component, in all cases, its concentration in the mixtures are unknown. Only two cases of intoxication with "pure" TCDD have been reported.

The story of the discovery of TCOD is by now well documented (Holmstedt 1980, Sandermann 1984a,b and Sandermann personal communication). TCDD was synthetized in 1955. Four people were intoxicated, one coworker severly while drying cristals. This man still suffers from persistent chloracne but continued to work at least until 1973 (last publication). 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 lymphglands. Ignorance of the toxicity of the compound that had been synthesized is proven by the following quotation:

> "The author placed an open crystallization dish with the chlorination products on his desk. After a certain time he experienced a severe irritation and skin reddening on the chin and comedones and pimples on the cheeks, the typical symptoms of a chloracne, as K.-H. Schulz later described it as a consequence of a TCDD illness. Some secondary symptoms were remarkable, such as fatigue, insomnia and noticable loss of memory. In consequence, the author had to interrupt frequently his lectures because his memory failed no connection with the chlorination product that was standing open on his desk was recognized" (Sandermann 1984a)."

In all cases the signs and symptoms dissapeared within a couple of years with the exception of the chloracne in the 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 had "transient minimal exposure to TCDD" are described. Two of them suffered from typical chloracne. Delayed symptoms about two years after initial exposure occurred in two of the scientists. These symptoms are said to have included personality changes, other neurological disturbances and hirsutism. All three scientists were found to have raised serum cholesterol but no other biochemical disturbances, and no porphyrinuria or liver damage was demonstrated. The question whether the unusually delayed physiological effects were in fact due to the initial dioxin exposure were discussed by the author. Although conclusive evidence is lacking, it seems likely that these delayed effect were in fact due to dioxin intoxication. The conditions of exposure remain unexplained.

In a attempt to follow up this case a telephone call to dr Norman Aldridge of the Toxicology Research Unit of the British MRC (1984-05-25) revealed the following:

Dr Muriel Brown, Senior officer in the cabinet office stated the following. All the subjects are now healthy. Only two are still employed by the Dept of Agriculture. They have been investigated quite a few times and are in fact fed up with the investigations. Their hirsutism and other skin symptoms have resolved. With regard to lab values liver function, cholesterol level, lipo-protein electroforesis, triglycerides in blood and porfyrins in urine are all within normal values.

There are three reports on delibrate application of TCDD or TCDD in mixture to the skin the firts one being the selfexperiments by Schultz (1961). KH Schultz wanted to prove the chloracnegenic property of TCDD in man and applied a 0.01% solution twice to a limited area of his lower arm. This caused a slight dermatitis after two days and later to hyperkeratosis and commedones. Chloracne was proven by excision and histology.

An experiment with volunteers was carried out by Kligman (Rove 1980, Hay 1982). Kligman then experimented with a higher concentration of dioxin on a further ten volunteers. This time the amount used was 7,500 µg of dioxin applied to the back in an area one inch square. A dose almost one thousand times higher. Not surprisingly perhaps, eight of the ten volunteers now

developed chloracne. The skin disease - which Lasted 4-7 months - was the only apparent medical complication these men suffered. The two series of tests merely show that a dose of somewhere between 16 and 7,500 µg of dioxins is required to cause chloracne in man - hardly an exact figure for a threshold dose (Hay 1982).

It has already been mentioned that Hoffman et al 1962 applied a TCP reaction mixture containing TCDD both to the skin of volunteers and to non affected skin areas of people who had already developed chloracne from this mixture.

Of the many cases of exposure reported in table 9.1 only two have been adequately followed up epidemiologically with matched control groups (Monsanto 1949 and BASF 1953).

The workers of Monsanto, USA have been investigated from one time to another between 1949 and 1984. Immediately after the accident Ashe and Suskind (1949) hospitalized and studied four cases of severe poisoning among the workers. They summarized;

> "It is our opinion that these men are suffering from systemic intoxication from a common agent arising out of their employment. This intoxication is characterized by acneform skin lesions, hepatitis, disturbed lipid metabolism, peripheral neuropathy and probably mild central nervous system involvement. From the point of view of the morphologic dermatologist, the type of exposure and the resultant skin lesions may justify the diagnosis of chloracne. There is little, if any, information reported concerning metabolic and systemic disturbances associated with chloracne. In the cases herein reported metabolic and systemic manifestations other than the skin were prominent. Their course to date suggests that there will probably be slow improvement in all manifestations with ultimate complete recovery. Three of the men have already recovered from symptoms of peripheral neuropathy. The skins of most of the men have improved, and may be expected to continue to improve rapidly under hygienic care when free from irritant atmospheres, as was evident during their stay in Cincinnati".

The four men who were initially examined October 1949 as well as two additional men were reexamined in 1950 (Ashe and Suskind 1950). Among signs and symptoms the following was noted:

> "The central nervous system symptoms have been those of irritability, nervousness and insomnia. Those examined have had consistently a loss of libido and some impotence. Hepatomegaly, tenderness and soreness in the right upper quadrant and epigastrium, in addition to a delayed prothrombin time, indicate a disorder of the hepatic tissue. Fasting serum lipids in eight out of nine subjects with clinical findings, are definitely increased. In four out of seven subjects there is an increase in total serum cholesterol. It is our considered opinion that the intoxication in each person affected has given rise to a disturbance of lipid metabolism which has affected several organ systems. The prognosis is excellent."

A total number of 36 workers from the same plant were examined in 1953 (Suskind et al 1953). At this time it was noted:

> "It is apparent from the medical histories and clinical findings that those who developed in moderate or severe degree the syndrome characterized invariably by an acneform eruption, pains in the extremities and back, dyspnea, fatigue, loss of vigor, nervousness, and decrease in libido, generally improved. Those who had the cutaneous eruption in its most severe and extensive form now have few or no lesions".

Laboratory studies including prothrombin time were now on the whole within normal limits.

More recent studies are those of Zack and Suskind (1980), Zack and Gaffey (1983), which cover both mortality and health. Zack and Gaffey report the following.

A 121-member study cohort, with a presumptive high-peak exposure to TCDD, was followed for mortality through 1978. The entire cohort was traced: thirty-two deaths were observed and eightynine persons were confirmed as living. Analysis indicated no excess in total mortality or in deaths from malignant neoplasms. The proportional mortality analysis of descendents by 2,4,5-T exposure classi-

fication indicated no unusual patterns of mortality in the 2,4,5-T exposed. 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 which was somewhat higher in the exposed group.

The Monsanto workers were again examined in 1984 (Suskind and Herzberg 1984). A clinical epidemiologic study was conducted to determine the long-term health effects of workplace exposure to 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. Among the exposed, clinical evidence of chloracne persisted in 55.7%. None of the not exposed 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 gastrointestinal tract ulcer. 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 after age adjustment at this time. The data assembled in the study indicated no evidence of increased risk for cardiovascular 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 TCOD exposure and were not therefore "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 outcome.

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 markedly improved 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 debil.itating 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). Twentyseven years after the accident which occurred in the BASF, Ludwigshafen plant a mortality study of persons exposed in the uncontrolled reaction during the trichlorophenol process was undertaken. The followup was 100% successful and involved 74 persons. 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 persons, 7 had cancer, compared with 4.1 expected. In addition, two other cases of cancer (one bronchial carcinoma, and one carcinoma of the prostrate) 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 only be explained as mere chance event. Of 74 persons, 66 had severe chloracne or severe dermatitis. Epidemiological studies usually cannot prove a casual relationship, but rather only indicate possible risks. 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 which 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 and epidemiological studies, a host of other follow-up studies have been published, none of which uses adequate controls. They are, therefore, of less value but will be briefly summarized here.

Jirasek et al (1973, 1974, 1976) and Pazderova et al (1974, 1980, 1981) examined 55 of a total of 80 workers who suffered intoxication during the manufacture of sodium pentachlorphenate and the sodium salt of butyl ester of 2,4,5-T. One worker died from severe acute intoxication at an early stage (Jirasek 1976), and 76 workers developed chloracne. The following additional symptoms were found: porphyria cutanea tarda, disorders of the lipid metabolism, porphyrins, carbohydrates and plasma proteins. Hepatic lesions were also present. Neurological, including electromyographic (EMG) examination, revealed peripheral nerve changes in 17 persons, first detected in 8 persons during the 2nd year of the study. A neurathenic 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 on very slowly. Unquestionally the workers in the Spolana factory were exposed to TCDD in a mixture.

> "The toxic substances were led off through the breathing zone of the workers".

"The concentrations of the above-noted chlorinated hydrocarbons in the air was never measured. Due to insufficient data, we cannot accurately reconstruct the real hygienic conditions at the work place. The manufacturing was definitively halted 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 pentachlorophenolate was manufactured. Not until 1965 was the manufacture of 2,4,5-TNa commenced on a pilot scale, and later that of the butylester of 2,4,5-T also. After each year of production, something was always changed or modified in the process and technology, frequently after several months, a certain part of the production was interrupted so that actually there was never a full-scale production in the true sense of the word. Many of the herbicides manufactured could not be found from the documentation".

The uncertain mixture of compounds involved in the Spolana episode makes interpretation of signs and sympoms next to impossible. In all Likelihood the porfyria observed was due to the hexachlorobenzene stated to be produced at this factory.

Another publication also mentions signs of disturbance in the porphyrin metabolism (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 could not be shown to differ from normal populations in most instances. In all likelihood the porfyria cutanea tarda in this case s in the previous is due to another compound than TCDD.

The original cases from nothern Germany were published by Bauer et al (1961). In their communication it is not quite clear where the cases originated and only 9 patients were studied in depth. These patients, plus an additional one, have been considered in a publication by Kleu and Göltz (1971) who summarized their findings in the following way.

> "The case histories of 10 patients who suffered from chloracne due to a professional trichlorphenol exposure were studied over a 15 year period. The patients developed a psychopathological syndrome, the intensity of which increased with the duration of the intoxication. The dominating complaints were decreased sexual activity, easy fatigability, muscular weakness, irritability, loss of appetite and memory, alcohol intolerance, discouragment, loss of interest. At present a permanent defect is manifesting itself with a reduction of vital, psychic and intellectual capacities independent of age. The late form resembles a cerebral involutionary syndrome combined with mental depression and neurasthenia".

Another follow-up study was published by von Krause and Brassow (1978). It appeared that in 1955, 24 workers at Boehringer had skin lesions. Twenty years later, in 1976, 11 of the original 24 workers were reexamined. Many continued to suffer from their earlier complaints. In seven of the 11, nausea and intolerance to heavy fatty food was still common. Six men complained of alcohol intolerance. Although conjunctivitis had disappeared, chloracne was still clearly visible in most of the group. Neurological problems were still severe in six of the workers.

Ten years after the incident at Coalite (1970) following which 79 workers developed chloracne due to exposure a study was undertaken to establish the state of health of the affected employees remaining in the company's employment (May 1982). The opportunity was used to examine effects on mortality, morbidity, carcinogenesis, reproduction, teratogenicity, fetotoxicity, biochemistry, immunology and genetic change. Concurrently, control groups were established with which to make comparison. The control groups selected from within the works matched the study group in respect of 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, there was no report that they had been adversely affected in any way. The many phases of this investigation have been critizised by Hay (1982).

The mortality data of workers at Dow Chemical company have been covered 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 a 1964 chloracne incident, presumably as a result of skin absorption of the process contaminant TCDD, 49 of these trichlorophenol production workers developed the skin condition. Within the limitations posed by cohort size and length of follow-up, the exposure 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 were statistically significant. The second paper examined the mortality experience of 204 persons exposed to 2,4,5-T during its manufacture from 1950 to 1971. Length of employment in job assignments within the 2,4,5-T process area ranged from less than one year to a maximum approximately ten years. Efforts to minimize TCDD contamination of the product resulted in non-detectable concentrations using a method of detection developed in 1966 that was sensitive to 1 part per million. Within the scope of this mortality survey, no adverse effects were observed with respect to occupational exposure to 2,4,5-T or its feedstock, 2,4,5-trichlorophenol.

The International Agency for Research on Cancer has evaluated the carcinogenic risk of TCDD to man (IARC 1977). For this evaluation, a number of cases of cancer, reported in workers exposed to TCDD, were available for review, included the one by Thiess 1982 and the liver tumours that had been reported from Hanoi. The IARC working group was unable to assess the significance of these reports because they were insufficiently detailed.

Hardell and his coworkers in Sweden have conducted 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 (thesis Hardell 1981). These authors also reported a case-control study suggesting that phenoxyacetic acids and chlorophenols may also predispose to Hodgkin's lymphoma (Hardell et al 1981). The relative risk was reported 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 but a risk increase 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.

In follow-up studies or workers exposed to 2,4,5-T and its precursor 2,4,5-trichlorophenol and presumable also TCDD no excessive deaths due to any cause was registered (Zack and Suskind 1980, Ott et al 1980, Cook et al 1980, Zack and Gaffey 1983).
Honchar and Halperin (1981) merged the 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) have 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 which have been reported (Cook 1981, Moses and Selikoff 1981, Johnson et al 1981). Three out of the seven cases had a record of chloracne and one of dermatitis. Upon review of the tissue specimens 5 of the 7 cases were diagnosed as soft-tissue sarcoma. The remaining two which also were part of the three cases in the merged cohort of Honchar and Halperin (1981) were found to be carcinoma. For three of the cases with confirmed soft-tissue sarcoma the exposure was found not to be documented although an undocumented contact with 2,4,5-T, 2,4,5-trichlorphenol or TCDD could not be excluded.

A cohort study on Swedish farmers and gardners in the period 1961-1979 did not reveal an increased incidence of soft-tissue sarcoma, Hodgkin's and non-Hodgkin's lymphoma (Eklund 1983).

10. Polychlorinated dibenzofurans

Polychlorinated dibenzofurans (PCDFs) are members of the chlorinated aromatic group of chemicals which includes the polychlorinated dibenzo-p-dioxins and the polychlorinated biphenyls. PCDFs have been identified as by-products in commercial PCBs, chlorinated phenols and chlorinated phenol-derived products....). Moreover, these compounds have recently been identified as by-products which are formed in the combustion or organic-containing wastes including municipal garbage. The major human exposure to PCDFs is associated

with the "Yusho" poisonings in Japan and Taiwan in which industrial PCB fluid, contaminated with PCDFs, inadvertently leaked into rice oil which was distributed and consumed by the victims. Several recent studies have confirmed the presence of PCDFs in numerous environmental matrices. The analysis and toxicology of PCDFs and related halogenated aromatics are complicated by the multiplicity of isomers and congeners which exist within each series. It is also apparent that their biologic and toxic potencies are remarkably dependent on structure.

10.1 Effects on animals

10.1.1 Chemobiokinetics and metabolism

PCDFs are absorbed from the gastrointestinal tract by passive diffusion across cell membranes. Prior to urinary and biliary excretion these highly lipid-soluble compounds need to be metabolically transformed into more polar derivatives. The less halogenated PCDFs are usually biotransformed and excreted more rapidly than the more halogenated ones. Thus, once absorbed the highly halogenated congeners may be more persistant and therefore may have a greater potential for bioaccumulation.
The rate of metabolism varies not only with molecular structure but also among species. It seems like the sensitivity to PCDF-poisoning is inversely related to the ability of a given species to metabolize and excrete the compound. In most species PCDFs are much more readily metabolized and excreted than their PCDD-counterparts. However the relation of toxicity to metabolism is complex apparently metabolism constituts a route of detoxication for PCDFs. PCDFs concentrate, not only in adipose tissues, but preferently also in the liver and other possible sites of action, however, the hepatic concentration of a PCDF-congener does not necessarily relate to its toxic potency.

10.1.1.1 Studies with 2,3,7,8-Tetrachlorodibenzofuran (2,3,7,8-TCDF)

The analog of TCDD and the most toxic isomer of the PCDFs 2,3,7,8-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 i.v. dose of 30.6 µg ^{14}C-2,3,7,8-TCDF/kg body weight was given to the

rats, mice and monkeys while the guinea pigs received an i.v. dose of 6 µg/kg. The distribution of the radiolabel 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 time-points studied is presented in table 10-1 along with the respective half-lives and LD5g-valus for 2,3,7,8-TCDF. It was shown that radioactivity recovered from the tissues represented the parent compound while radioactivity in feces and urine represented metabolites of 2,3,7,8-TCDF, however, in the feces of guinea pigs only the parent substance was present. TLC-analysis revealed R_f-values of 0.5 and 0.1 for metabolites of 2,3,7,8-TCDF in feces and urine as compared to R_f-0.8 for the parent compound. 2,3,7,8-TCDF has a short half-life, 2 to 4 days, a high LD_{50}-value, > 6 000 µg/kg, and is quickly eliminated from the liver both in the rat and the mouse. Elimination occurs rapidly also from the skin and muscle whereas retention is longer in adipose tissues. The difference in retention of 2,3,7,8-TCDF in adipose tissues between C57Bl/6J and OBA/2J mice can be explained as due to the fact that DBA/2J mice have substantially more adipose tissues than C57Bl/6J mice.

The distribution of 2,3,7,8-TCDF in the guinea pig was completely different from that in the rat or mouse. The maximum uptake in the liver occurred within one hour after dosing, after which time the radioactivity was distributed in the fat and skin during the succeding hours. After one day, in connection with 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 slightly. The estimated half-life for 2,3,7,8-TCDF in the guinea pig was more than 20 days.

Based on data from 3 monkeys the half-life for 2,3,7,8-TCDF was calculated to 8 days. At the end of the study more radioactivity remained in adipose tissues and skin than in the liver. The retention of 2,3,7,8-TCDF in the liver of monkeys 21 days after dosing was comparable to that in the liver of the rat and C57Bl/6J mouse 10 days after injection.

Urinary elimination of radioactivity was a minor route when compared to fecal elimination both in the rat, mouse and monkey whereas in the guinea pig these routes were of comparable sizes. The cumulated excretion of radioactivity 3 days post-treatment amounted to about 64%, 51%, 11% and 7% in the rat, C57Bl/6J-mouse, monkey and guinea pig respectively.

Against this background of the data on tissue distributions, half-lives and LD_{50}-values of 2,3,7,8-TCDF in the rat, guinea pig and monkey Birnbaum et al (1981) concluded that metabolism of 2,3,7,8-TCDF, measured as excreted radioactivity, is a detoxification route and that animal species with a high capacity to metabolize 2,3,7,8-TCDF thus are more resistant to its acute toxicity. This conclusion was considered applicable also for the mouse (Decad et al 1981b). There are certain objections against the basis of this comprehensive conclusion. First, the kinetic studies on guinea pigs (Decad et al 1981a) was for analytical reasons carried out with such a high dose of 2,3,7,8-TCDF, that all the animals showed marked signs of toxicity even within 3 days. After 9 days all the animals were killed due to the toxic symptoms. It is not advisable to draw any conclusions on normal kinetic behaviour from data obtained on dying animals with their unnormal metabolism and physiology. As far as the kinetic data from the monkey are concerned the conclusions are based on a single time-point, furthermore, the number of animals in that study is very limited (Birnbaum et al 1981).
Thirteen chlorinated compounds were detected in bile collected for 48 h from female Sprague-Oawley rats given a single oral dose of 678 µg 2,3,7,8-TCDF (79.4% pure)/kg body weight (Poiger et al 1984). The four major metabolites considered to originate from 2,3,7,8-TCDF were trichloromethoxy-, two trichlorodimethoxy- and tetrachloromethoxydibenzofuran. The remaining nine metabolites, detected in minute amounts, likely originated from contaminating PCDFs (1% of tri-CDF, 8.4% tetra-CDFs, 11.2% penta-CDFs).

10.1.1.2 Studies with other PCDF-congeners

A mixture of PCDFs, 2 tetraCDFs, 4 pentaCDFs and 4 hexaCDFs, was given as a single i.p. injection of 500 yg to male ICR mice (Morita and Oishi 1977). The distribution pattern of the isomers in various tissues were followed for up to 8 weeks. Analysis were performed with GC-EC 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 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 dissapeared from the liver. Isomers with longer retention times were slowly absorbed thus they appeared

Later and then persisted for Long time 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.

Yoshihara et al (1981) administered single i.p. injections of 1, 5 or 10 µg/kg of 13 individual PCDF congeners to young male Wistar rats. 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 less accumulative, however, three of the six isomers having no vicinal hydrogens (1,3,6,8-TCDF, 2,3,7,8-TCDF and 1,2,4,6,8-PCDF) also showed low accumulation. The most highly accumulative isomer 2,3,4,7,8-PCDF, was retained to more than 65% of the dose, whereas 2,3,7,8-TCDF, which is equally biologically potent, was retained to 3.8% only. These results would imply that there is no relationship between hepatic distribution and potency of acute toxicity of PCDFs. Animals in this study were strongly induced and showed toxic symtoms, except for the following isomers: 2,8-DCDF, 1,2,7,8-TCDF, 1,3,6,7-TCDF, 1,3,6,8-TCDF, 1,2,4,6,8-PCDF. This is important to take into consideration when judging the kinetic data. A mixture of 14% 1,2,7,8-TCDF, 35% 2,3,7,8-TCDF, 1% 1,2,4,7,8-PCDF, 49% 1,2,3,7,8-PCDF, 1% 2,3,4,7,8-PCDF and 1% hexaCDF was administered as a single i.p. 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).

Metabolites of 2-MCDF, 2,8-DCDF, 2,3,8-TCDF and OCDF was determined in urine, feces, fat and liver of male Wistar rats given a single oral dose of 250 mg/kg body weight of the respective isomers (Veerkamp et al 1981). Analysis were performed with GC-MS. No metabolites in any samples were found in rats given OCDF. Mono- and dihydroxyderivatives were obtained with all other isomers whereas sulphurcontaining metabolites were detected only with the mono- and dichlorodibenzofurans. Metabolites from 2-MCDF and 2,3,8-TCDF were found in urine and feces only, but with 2,8-DCDF metabolites appeared also in the tissues.

10.1.1.3 Transfer of PCDFs via placenta and/or milk

Nagayama et al (1980) studied the transport of a mixture of PCDFs through placenta and mother's milk in mouse. A diet containing 0.6 ppm PCDFs (48% tetraCDFs, 49% pentaCDFs and 3% hexaCDFs) was given for 18 days after mating. The placenta transport calculated from the amount of PCDFs in the neonates was ca 0.003% of the administered dose. The isomers that remained were 2,3,7,8-TCDF and 2,3,4,7,8-PCDF. After giving birth, the mothers were fed a diet free from PCDFs. While the whole-body levels of PCDFs in the mothers sunk from 5.5% to 1.6% of the dose, the whole-body levels in the suckling young ones increased from 0.003% at the time of birth to 0.07% after one week and to 0.14% after 2 weeks. 2,3,7,8-TCDF and 2,3,4,7,8-PCDF were the dominant species present in both the mothers and the pups. To study the transport through milk only, the same diet was given to pregnant rats from day 18 after mating and 14 days further when the young ones suckled. After 14 days 5.1% of the administered dose was found in the mother. In the young ones 0.3% and 1.2% of the given dose was found after 1 and 2 weeks, respectively. The dominant isomers recovered in the offspring were 2,3,7,8-TCDF, 2,3,4,7,8-PCF and one unidentified pentaCDF i.e. the same isomers found in largst amount in the mothers liver. The data demonstrated that the amounts of PCDFs transferred through milk were much larger than the amounts transferred across the placenta.

10.1.2 Acute toxicity

Single oral LD_{50}-values for 2,3,7,8-TCDF in various species are listed in table 10-2.

10.1.2.1 Studies on rats

Intravenous administration of 30.6 µg TCDF/kg body weight to male Fisher rats caused listlessness, excessive hairloss 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 nor of liver hypertrophy (Birnbaum et al 1980). Single i.p. injection of 1 or 10 mg/kg body weight of 9 individual PCDF

isomers, having at least three chlorines in the lateral positions, to male Wistar rats were able to produce thymus atrophy and liver hypertrophy 5 days post-treatment. The most potent isomers 2,3,7,8-TCDF and 2,3,4,7,8-PCDF were effective allready at a single dose of 1 µg/kg. Five other congeners having two or less chlorine in the lateral positions did not cause any effects on thymus or liver when given in the same dose range (Yoshihara et al 1981).

10.1.2.2 Studies on mice

Moore et al (1976) failed to establish a lethal dose for 2,3,7,8-TCDF in C57Bl mice when giving single oral doses of up to 6 000 yg/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 6 000 µg 2,3,7,8-TCDF/kg was given s.c. Poland and Glover (1980) found 2,3,7,8-TCDF-induced thymus atrophy in C57Bl/6 mice 5 days after a single i.p. dose of $3x10^{-7}$ moles/kg body weight. Single doses of 100 to 1 000 yg 2,3,7,8-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 2 tetraCDFs, 4 pentaCDFs and 4 hexaCDFs given as a single i.p. dose of 500 yg to ICR mice produced no lethality within 8 weeks (Morita and Oishi 1977). CF-1 mice given a PCDF mixture containing 42% tetraCDFs, 54% pentaCDFs and 4% hexaCDFs as a single oral (10 to 1 000 mg/kg), s.c. (10 to 200 mg/kg) or i.p. (10 to 100 mg/kg) dose developed no toxic symptoms during the first week except a modest weight loss (Nishizumi et al 1978). The first deaths occurred 8 days after an oral dose of 1 000 mg/kg, 5 weeks after a s.c. dose of 200 mg/kg and 11 days after an i.p. dose of 100 mg/kg. The oral LD_{50-30} was calculated as 184 mg/kg for males and 414 mg/kg for females. In mice that died hepatomegaly and thymus atrophy were consistent findings. In surviving mice on high dosages the liver exhibited small necrotic foci accompanied by cellular infiltrates. The hepatic leisons occurred in centri-lobular areas and enlarged hepatocytes containing foamy cytoplasm, increased numbers of lipid droplets and proliferation of smooth endoplasmatic reticulum were seen.

10.1.2.3 Studies on guinea pigs

The pattern of toxicity was similar for 2,3,7,8-TCDF, 2,3,4,7,8-PCDF and 2,3,7,8-TBrDF when given to young Hartley guinea pigs (Moore et al 1979). The single oral LD_{50} was >5 <10 µg/kg for all three isomers and the average time to death was 12 to 15 days. Overt signs at lethal doses were characterized by immediate and progressive weight loss, rough soiled hair coat, listlessness and dehydration. Similar symptoms appeared 3 days after an i.v. injection of 6 µg 2,3,7,8-TCDF/kg (Decad et al 1981a). At necropsy lack of body fat, reduced body mass and thymus weight were found. Histologic findings were primarily associated with deplation of lymphoid cells in thymic cortex but hypocellularity of bone marrow and hyperplasia in epithelial cells of renal pelvis, uretar and urinary bladder were also observed. Liver leisons were not observed. Surviving animals showed mild thymus lymphoid hypoplasia only. Sublethal doses resulted in decreased body weight gain.

10.1.2.4 Studies on monkeys

The single oral LD_{50}-value for 2,3,7,8-TCDF in the young female Rhesus monkey (Macaca mulatta) was determined to 1 000 µg/kg in a 60 days study with the dose-range 0, 500, 1 000 and 1 500 yg/kg and with 2 or 4 animals at each dose-level (Moore et al 1979). The two monkeys that received the sublethal dose developed skin leisons and had a decreased body weight gain. With lethal doses the following overt symptoms occurred after 7 to 10 days: progressive weight loss, loss of body fat, facial edema, loss of facial hair and 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 leisons were observed though the liver weight was increased. Increases in serum albumin and cholesterol were also recorded. Three male Rhesus monkeys (Macaca mulatta) given the very low single dose of 30.6 yg 2,3,7,8-TCDF/kg body weight did not gain weight the three following weeks and they developed facial skin leisons preferently of sebaceous glands (Birnbaum et al 1981).

10.1.2.5 Studies on rabbits

Bauer et al (1961) demonstrated the occurrance of severe and even fatal Liver necrosis in rabbits exposed to a mixture of triCDFs and tetraCDFs in a single oral dose of 0.5 or 1 rag/kg body weight. Dermal application of the same mixture resulted in severe hyperkeratosis at the application site.

10.1.3 Subchronic toxicity

10.1.3.1 Studies on rats

Male SD rats were fed 1 or 10 ppm of a PCDF-mixture, containing 2 tetra-CDFs, 4 pentaCDFs and 4 hexaCDFs for 4 weeks (Oishi et al 1978). Decreases in growth, food consumption, hemoglobin and hematocrit values, erythrocyte counts, serum levels of triglyceride, testosterone, glutamic pyruvic transaminase and leucin aminopeptidase activities as well as increases in serum cholesterol, cholinesterase and gutamic oxaloacetic transaminase activities were demonstrated with both diets. Rats fed the 10 ppm diet developed chloracne-like leisons 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. Additionally, in a similar study no effects were seen on total serumproteins or leucocyte counts (Oishi et al 1977).

10.1.3.2 Studies on mice

C57Bl/J67 mice given 2,3,7,8-TCDF orally 5 times weekly for 30 days did not develop clinical signs of toxicity in any of the dose-groups of 30, 100 or 300 µg/kg body weight. Thymus atrophy, liver hypertrophy, decreased leucocyte count and slightly elevated total serum protein occurred in the high dose group at the end of the study (Moore et al 1979). Daily doses of 10, 30 and 50 µg 2,3,7,8-TCDF/kg body weight on gestation days 10 to 13 produced a dose related increase in maternal liver weights (Weber et al 1984).
Decreased thymus weight was recorded in ICR/JCL mice exposed to 4 weekly doses of 100 µg/kg of a mixture of 12% tetraCDF and 88% pentaCDF (Oishi and Hiraga 1980). When 0.6 ppm PCDFs of unknown composition were given in the diet to

mice for 10 weeks severe dermal lesions with hyperkeratosis and dilated hairfollicles filled with keratinous material occurred in 7 out of 12 mice. Furthermore, hepatocytes had enlarged nucleus and vacuolations in the cytoplasm (Nagayama et al 1979). Feeding female ddN-mice 0.6 ppm PCDFs (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 nor in offspring (Nagayama et al 1980).

10.1.3.3 Studies on guinea pigs

Four adult male Hartley guinea pigs were given 6 to 7 weekly doses of 1 µg 2,3,7,8-TCDF/kg body weight (Decad et al 1981a). The animals started to loose weight rapidly after the 5th or 6th dose i.e. 30 days after the first dosage when the cummulative dose reached 5 or 6 µg/kg, 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 administration of low letal doses of 2,3,7,8-TCDF accumulate and may lead to a critical body burden which will result in an irreversible and progressive weight loss eventually followed by death. 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 dosage group (Luster et al 1979). The thymus weight was decreased in the 0.5 and 1.0 ug/kg dosage 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.

10.1.3.4 Studies on monkeys

A two month study with 3 young male Rhesus monkeys (Macaca mulatta), serving as their own controls, fed a diet with 50 ppm 2,3,7,8-TCDF resulted in one case of illness after 1 month and one death after 2 months when the cumulative dose was calculated to 300 ug/kg (McNulty et al 1981). Toxic changes observed after 1 month included periorbital edema, reddening and thickening of the eye-lids, enlargement of facial hair follicles and decreased number and size of sebaceous glands in the skin. After 2 months these changes had progressed

severly and were accompanied by decreased physical activity and elevated, or eventually lost, toe- and fingernails. 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 the behaviour, clinical appearance and histological structure of the skin were normal. The monkey that died had lost 23% of its initial weight and body hair were almost gone. Sebaceous glands were replaced by small squamous cysts. Severe leisons were confined to the skin, thymus and the stomach epithelium whereas liverleisons were modest.

10.1.3.5 Studies on rabbits

The 25% etherhexane extracts from two commercial polychlorinated biphenyl preparations (PCB) 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. Liverleisons 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 and Beems 1971). A mixture of tetraCDFs and pentaCDFs was much less potent in producing hyperkeratosis when applied to the inside of depilated rabbits ear for 3 consecutive days than was TCDD (Nishizumi et al 1975).

10.1.3.6 Studies on chickens

Mortality in one day old white Leghorn chicken given 1 or 5 µg 2,3,7,8-TCDF/kg body weight orally for 3 weeks were 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 edema, ascites and hydropericardium as well as thymus atrophy occurred. Depletion of lymphatic cells was evident both in the spleen and thymus. Mild liver leisons were found only in the high dose-group. Total serum protein and serum albumin were reduced.
The significant difference in toxicity between three commercial polychlorinated biphenyl(PCB) preparations in chickens (Vos and Koeman 1970) was later demonstrated to be caused by the presence of tetra- and pentachlorodibenzo-

furans in two of the three preparations (Vos et al 1970). The 25% ether-hexane extracts from these two preparations were highly toxic in the chick embryo assay (Vos et al 1970) whereas no effect could be produced by the ether extract from the third preparation lacking PCDF-impurities.

10.1.4 Chronic toxicity

10.1.4.1 Studies on monkeys

Three young male rhesus monkeys (Macaca mulatta) were exposed to 5 ppb of 2,3,7,8-TCDF in the diet for 6 months. One animal served as a control (McNulty et al 1981). 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 2,3,7,8-TCDF caused symptoms similar to those following a single large dose of 2,3,7,8-TCDF (see 10.1.2.4) or acute or chronic ingestion of TCDD (see 8.1.1 and 8.3). The major histopathological effects 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 2,3,7,8-TCDF when chronically ingested was approximately equal to that of TCDD. This is in contrast with the acute toxic effect of 2,3,7,8-TCDF which is approximately 20 times less than that of TCDD. The reason for death in the TCDF-poisoned monkeys was obscure. Death was preceeded by weight loss, anorexia and depression. Only modest thymic and epithelial changes and no evidence for liver damage were present. 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.

10.1.5 Immunobiological effects

Studies on immunobiological effects of PCDFs have not demonstrated severe immunosuppressive effects in adult animals exposed to sublethal doses. Only with doses which induce overt toxicity is there a pronounced effect.

Similar modest immunosuppressive effects were also found with TCDD when studies were performed in adult animals. It was not until TCOD was administered during the developmental phase of the immune system that the consequences of TCDD on the immunesystem were recognized. Hitherto no studies have been performed on the effects of PCDFs on the developing immune system. Comparative studies on humoral immunity responses in mice have revealed that 2,3,7,8-TCDF produce a similar pattern of responses as do TCDD but at 30 times higher doses. Furthermore the immunosuppressive effect of TCDD is much more persistant (Vecchi et al 1983b).

10.1.5.1 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 (Moore et al 1979, Nishizumi et al 1978), rat (Oishi et al 1977, 1978), guinea pig (Moore et al 1979) and monkey (McNulty et al 1981, Moore et al 1979). Also in studies aimed at investigating immunobiological effects have decreased thymic weights been reported (Luster et al 1979, Vecchi et al 1983b). The histological findings are similar to those occuring after TCDD-exposure i.e. loss of lymphoid cells in thymic cortex. A reduced number of spleenocytes was obtained from mice treated with 180 µg 2,3,7,8-TCDF/kg (Vecchi et al 1983a) but no spleenic pathology was reported in mice given A weekly doses of 100 µg/kg of a PCDF-mixture containing 12% tetraCDFs and 88% pentaCDFs (Oishi and Hiraga 1980). Peritoneal cell and macrophage counts were not modified by a 2,3,7,8-TCDF dose of 180 ug/kg to mice (Vecchi et al 1983b).

10.1.5.2 Humoral mediated immunity

Adult female Hartley guinea pigs exposed to 0.05, 0.17 and 0.5 µg 2,3,7,8-TCDF/kg body weight once weekly for 6 weeks showed somewhat depressed serum IgG concentrations. A dose-related depression in splenic lymphocyte proliferation after stimulation with 50 µg of the B-lymphocyte mitogen E. coli 0127 Upopolysaccharide/ml medium was seen in 2,3,7,8-TCDF-treated animals. 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 1979). The antibody response to sheep red blood cells given 7 days after *a*

single i.p. injection of 180 yg 2,3,7,8-TCDF/kg body weight was inhibited by 85% and 355! in C57Bl/6 and DBA/2 mice respectively (Vecchi et al 1983a) whereas a dose of 10 yg 2,3,7,8-TCDF/kg body weight to C57Bl/6 mice had no effect (Rizzardini et al 1983). The suppression was dose-dependent as well as time-dependent (Vecchi et al 1983b). By day 42 post-treatment a near normal antibody response was obtained.

10.1.5.3 Cellmediated immunity

Oral intubation of 0, 10 and 100 yg PCDF (12% tetraCDFs and 88% pentaCDFs)/kg body weight once weekly for four weeks increased the mortality dose-dependently in ICR/JCL mice after an i.p. injection of 0, 50, 250 and 500 yg endotoxin/mouse two days after the final treatment with PCDF (Oishi and Hiraga 1980). Only at high dose levels were there any effects on cellmediated immunity functions in female Hartley guinea pigs given 0.05, 0.17, 0.5 and 1.0 yg 2,3,7,8-TCDF/kg body weight once weekly for six weeks (Luster et al 1979). Both the depression in delayed hypersensitivity response to PPD and in the ability of BGG-sensitized lymphocytes to release the macrophage inhibition factor were related to the dose of 2,3,7,8-TCDF. Splenic lymphocytes, from 2,3,7,8-TCDF-treated animals,stimulated with the T-lymphocyte mitogen phyto-hemagglutation (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 2,3,7,8-TCDF-related effect. The increased proliferative response to Con A and PHA in thymocytes cocultivated with thymus epithelial (TE) cells or cultivated in TE-conditioned medium was inhibited if the TE-cells were pretreated with 2,3,7,8-TCDF for 48 h thus suggesting a direct effect on TE cells. (Osborne et al 1984).

10.1.6 Enzyme induction

PCDFs are potent enzyme inducers. Studies on 36 individual PCDFs have revealed that the enzyme inducting potency vary greatly depending on the position as well as the number of chlorines substituted. The structure-activity relation-ships of the PCDFs are similar to those for PCDDs with 2,3,7,8-TCDF and 2,3,4,7,8-PCDF being the most potent.

10.1.6.1 Studies on rats

Three daily doses of 2.5 µg 2,3,7,8-TCDF/kg body weight induced AHH- and UDPGT-activities, 38- and 3-fold respectively in female CD rats 24 h after dosing. The cyt P-450 content was dubbled whereas no effect was found on the aminopyrine N-demethylase activity (Goldstein et al 1978). Yoshihara et al (1981) gave a single i.p. dose of 1, 5 or 10 mg of 13 individual PCDF:s/kg body weight to young male Wistar rats five days prior to the determination of hepatic enzymeactivities. Congeners having at least three chlorine atoms in the lateral positions typically showed increased AHH- and DT-diaphorase activities while those congeners having two or less chlorine in these positions were not inductive. The cytochrome P-448 content was increased by 5 among the 13 congeners whereas the benzphetamine-N-demethylase activity was depressed by 7 among the 13. The most potent isomers 2,3,7,8-TCDF and 2,3,4,7,8-PCDF were effective allready at a single dose of 1 ug/kg body weight. The ranking of the inducing abilities did not coincide with the hepatic distribution of the substances. Hepatic AHH-activity in male Wistar rats was significantly enhanced only by 2,3,7,8-TCDF and 2,3,4,7,8-PCDF among 15 individual PCDF isomers when the administered dose was 5 ug PCDF/kg body weight (Nagayama et al 1983). Eight among 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 (1984a) investigated the effect of 3 tetraCDFs and 3 pentaCDFs at the dose of 500 and 1 000 ug/kg body weight on hepatic AHH-, aminopyrine-N-demethylase-, 4-chlorobiphenylhydroxylase- and ethoxyresorufin-O-deethylase activities in male Wistar rats. The most active compounds, 2,3,7,8-TCDF and 2,3,4,7,8-PCDF were potent inducers of the cytochrome P-448-dependent mono-oxygenases. Some induction of microsomal AHH, ethoxyresorufin-O-dethylase and 4-chlorobiphenylhydroxylase was observed also for the 2,3,4,8-TCDF and 1,2,4,7,9-PCDF.

A mixture of PCDFs, reconstituting the approximate composition found in human liver of Yusho victims, consisting of 7.4% 2,3,7,8-TCDF, 6.1% 1,2,4,7,8-PCDF, 19.0% 1,2,3,7,8-PCDF, 29.4% 2,3,4,7,8-PCDF and 39.1% 1,2,3,4,7,8-HCDF by weight, was given as single i.p. injections to male Wistar rats 14 days before measuring the induction of cytochrome P-448 related enzymactivities (Bandiera

ethoxyresorufin-0-deethylase activities were found in the range 10 to 400 yg PCDF-mixture/kg body weight.

10.1.6.2 Studies on mice

No induction of cytochrome P-448 content or of 7-ethoxycoumarin-0-deethylase activity were found 12 days after a single i.p. injection of 10 yg 2,3,7,8-TCDF/kg body weight to male C57Bl/6J mice (Rizzardini et al 1983).

10.1.6.3 Studies on chicken

Hepatic AHH-activity in chick embryos was inducible by 2,3,7,8-TCDF, 2,3,4,7,8-PCDF and 1,2,3,7,8-PCDF with ED_{50} values of 0.015, 0.014 and 0.071 nmole/egg (Poland et al 1976). No induction was produced by dibenzofuran, 2,8-DCDF, 2,4-OCDF, 2,4,8-TCOF or 1,3,6,7-TCDF with 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 chicken 3 weeks after treatment with a single oral dose of 1 yg 2,3,7,8-TCDF (Goldstein et al 1976).

10.1.6.4 Studies on cell cultures

Bandiera et al (1984a) studied the induction potencies of 26 isolated PCDF isomers in rat hepatoma H-4-II-E cell cultures. AHH- and ethoxyresorufin-0-deethylase activities were determined after exposing the cells to optimal doses of PCDFs for 5 days. Dibenzofuran, 2- and 3-chlorodibenzofuran did not induce these enzyme activities. EC_{50} values for all the remaining congeners varied between 1.0×10^{-5} M and 1.3×10^{-10} M. The most active inducer was 2,3,4,7,8-PCDF.

Human lymphoblastoid cell lines, derived from peripheral blood of healthy volunteers, both male and female, of various ages were exposed to 8 individual PCDF isomers for 48 h (Nagayama et al 1985). The AHH-inducibility was highly

variable between persons but less variable between isomers. In this system 2,3,7,8-TCDF was about half as potent as 2/3,4/7/8-PCDF, 1,2,3,4,6,7-HCDF and 1,2,3,4,7,8-HCDF which were equally potent as TCDD in AHH-induction.

10.1.7 Receptor binding

The competitive binding of PCDFs to the TCDD receptor protein has been studied in vitro both in the hepatic cytosol (Bandiera et al 1984a, Poland et al 1976) and nucleus <Poellinger et al 1982). Poland et al (1974) investigated the ability of 7 PCDF congeners to compete with TCDD in binding to the hepatic cytosol receptor from C57Bl/6J mice. They found the relative binding affinities for 2,3,7,8-TCDF, 2,3,4,7,8-PCDF and 1,2,3,7,8-PCDF to be 37%, 34X and 38% of the binding affinity between TCDD and the receptor. The EC_{50} values for the competitive binding of 26 individual PCDFs to the receptor from rat hepatoma H-4-II-E cell cultures varied from less than 10^{-3} M for 4-chloro-dibenzofuran to 1.5×10^{-8} M for the most active competitor, 2/3/4,7,8-PCDF/ which had an EC_{50}-value comparable to that for TCDD, 1.0×10^{-8} M (Bandiera et al 1984a). 58% of the TCDD bound to the nucleic receptor in vitro was displaced by a 100-fold molar excess of 2,3,7,8-TCDF. Nuclei were isolated from the liver of Sprague-Dawley rats pretreated with 1 µg 2,3,7,8-TCDF 2 h prior to the incubation (Poellinger et al 1982).

10.1.8 Teratogenicity and reproductive effects

It has been demonstrated that 2/3/7,8-TCDF is a potent teratogen in mice at doses which produce no overt toxic effects in dams. Malformations observed include cleft palate and kidney malformation, similar to hydronephrosis. Dose-related increase in fetal mortality occur with single high doses. The teratogenic pattern of 2/3,7/8-TCDF thus is strikingly similar to that of TCDD (see 8.5).

A diet containing 0.6 ppm PCDFs (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 (Nagayama et al 1980). Single doses of 100 to 1 000 µg 2/3/7/8-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. Palatal closure in mice occure late on day 14 of gestation thus 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 is somewhat conflicting in the two existing studies. Weber et al (1984) reported that 95.5% of the fetuses had kidney malformations after a dose of 500 yg/kg body weight on day 10 of gestation. While only 17% of the fetuses/dam had this malformation after a single dose of 400 yg/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 2,3,7,8-TCDF-induced kidney malformations, up to a certain degree, represents a reversible defect since no hydronephrotic kidneys were found in neonatals whereas in identically treated dams examined on day 18 of gestation over 80% of the fetuses/litter were affected. Fetal mortality increased dose-relatedly 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).

Recombinant inbred strains of C57Bl/6J and DBA/2J mice segregating at the Ah-locus, respond differently to the teratogenic effect of 2,3,7,8-TCDF (Hassoun et al 1984c). Fetuses of Ah-responsive strains respond with a high frequency of cleft palates and kidney malformations after a single i.p. dose of 600 yg 2,3,7,8-TCDF/kg body weight on day 12 of gestation. No cleft palates and only modestly increased numbers of kidney malformations in a few strains were found with the same treatment of Ah-nonresponsive strains.

10.1.9 Mutagenicity

No mutagenic activity was recorded for 2,9-DCDF, 3,6-DCDF, 2,3,7,8-TCDF or OCDF when the strains TA 98 and TA 100 were tested over a 3 log dose range in Ames reversion assay in the presence or absence of hepatic S9-fraction from normal or chemically induced rats (Schoeny 1982). Toxicity for the salmonella, particularly for TA 100, was noted only at 1 yg 2,3,7,8-TCDF/plate. The toxic effect was eliminated by the addition of S9.

10.1.10 Carcinogenicity

The hepatic tumor-promoting activity of a commercial polychlorinated biphenyl mixture, Aroclor 1254 with (Ar 1254) or without (Ar 1254-PCDF) its intrinsic polychlorinated dibenzofuran 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 ppm of Ar 1254 (≥ 3 ppm PCOF) or Ar 1254-PCDF (< 0.1 ppm PCDF) for 18 weeks. Light microscopic examinations of liver leisons demonstrated that Ar 1254 promotes production of hepatocellular carcinomas in rats, the promoting incidence (64%) remained essentially unchanged (84%) when PCDF was removed from Ar 1254 with 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 trace.

10.2 Human data

Several accidental ingestions of chloracnegenic compounds have occurred. They are of particular importance in relation to discussions wether chloracne is a systemic or local disease.

Already in 1947 Herzberg described several cases of chloracne and other signs and symptoms due to consumption of "chlorinated paraffin" used as a substitute for butter during cooking in post war 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, commedones and retentioncyst in sebaceous glands. It was noted as remarcable that the skin signs had a follicular predilection like in seborrhoe (face, head, bosom and back). The slow development of chloracne and particularily the affection of the sebaceous glands led the author to conclude that it was a secretory disease (Ausscheidungstoxikose). It is difficult to see how it could be anything else because no local contamination was recorded.

With regard to the chloracnegenic component it is unlikely that paraffine itself was active. Herzberg speculated that something else, may be a pyrolysis product due to heat during cooking, could have caused the disease.

A German preparation "Carbolineum" (German patent Nr 46021, 1888 and 542593, 1930) was manufactured by company R Avenarius. It was obtained through chlorination of a coaltar fraction that was known to contain a few percent of dibenzofurans, naphtalins, and bifenyls etc.

Braun 1955 was the first one to mention chloracne due to chlorinated dibenzofurans (p 24 in thesis), this was experimentally proved by Bauer et al 1961 and in 1970, Vos et al identified by mass spectrometry the occurrence of chlorinated dibenzofurans in commercial PCB-mixtures.

A mass outbreak of food poisoning occurred in western Japan 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.

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 also found to be contaminated with a smaller quantity of polychlorinated dibenzofurans (Nagayama et al 1975) and a relatively large amount of polychlorinated quaterphenyls (Miyata et al 1978, Kamps et al 1978, Masuda et al 1979).

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). At the end of 1980, the total number of reported cases was about 2000.
The levels of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) in the toxic rice oils which caused PCB poisoning in Taichung were determined by ECD-Gas Chromatography and Gas Chromatography/Mass

Spectrometry. The presence of polychtorinated quaterphenyls (PCQs) and polychlorinated terphenyls (PCTs) in the Chinese toxic rice oil has been published (Chen et al 1981).

The amounts consumed and the chemical composition of the various rice oils is given in table 9-4 and 9-5.
It has been discussed what materials in the rice oil gave rise to the signs and symptoms of Yoshu and Yu cheng (Japan-US Joint Seminar 1983 and Kuratsune and Shapiro 1984).
The data available so far can be summarized as follows.

PCDFs are found in rice oil and in tissues of Yusho and Yu-cheng patients. PCBs and PCQs fail to cause toxic signs similar to those of PCDFs in monkeys and rats. The PCDF-isomers that are retained in patients are retained in experimental animals and have a high biological activity.

PCDFs are selectively retained in the liver. PCB-levels correspond to fatty level of the tissue. PCDFs are not found in unexposed controls nor in PCB-exposed worker. PCB-levels in patients were only about twice higher than those of normal persons several years after the outbreak. PCB-exposed workers had more than 10 times higher PCB-blood levels than patients. PCQ-levels were similar.

Generally a correlation between degree or severity of clinical signs and the amount of PCDFs retained in blood was obtained. There is a lack of correspondence between disease severity and PCB-concentration in blood.
Mild dermal leisons seen in workers exposed to PCB disappeared quickly after discontinuation of PCB-handling contrary to the persistance of Yusho and Yu-cheng. Everything thus speaks in favour of the contaminant PCDFs being the causative agent.

The symptomatology of Yusho has been summarized by Reggiani 1982 and is to be found in tables (2 st) 9-6 and 9-7. They correspond on the whole to what has been described in the other episodes of intoxication in this chapter. However, there are differences such as the frequency of transient visual disturbance, hearing difficulties and a persistent bronchitis.

11. EVALUATION OF HEALTH RISK FROM THE EXPOSURE TO CHLORINATED DIBENZO-P-DIOXINS (PCDDs) and dibenzofurans (PCDFs).

CAUTION

THIS IS A TENTATIVE SKELETON DRAFT THAT WILL BE DISCUSSED BY THE TASK GROUP. IT DOES NOT CONSTITUTE THE FORMAL VIEW OF EITHER WHO OR THE AUTHORS OF THE PRESENT DRAFT OF THE CRITERIA DOCUMENT.

11.1 Introduction

The PCDDs and PCDFs constitutes a group of chemicals that by now have been demonstrated to occur ubiquitus in the environment. They do not occur naturally, nor are they commercially produced.

The sources of PCDDs and PCDFs so far identified are mainly the following:
a) contaminants in certain commercial chemicals, e.g. TCDD in 2,4,5-trichlorophenol and products derived from it such as the phenoxyacetic acid 2,4,5-T. While TCDD is generally present in low concentrations in commercial products, waste material generated in the production of these compounds may contain very high concentrations of TCDD (up to 1 000 ng/kg or more). However, even the low concentration of TCDD in commercial products (less than 1 mg/kg) are of concern.
b) Contaminants in commercial mixtures of polychlorinated biphenyls (PCBs). PCBs have been demonstrated to contain high levels of PCDFs (table 4-6).
c) Synthesis by pyrolysis of various waste material. Depending on the composition of the material burned and the conditions of the incineration varying amounts of the differents PCDDs and PCDFs are formed.

The PCDDs and PCDFs are two series of almost planar tricyclic aromatic compounds with very similar chemical properties. There are 75 congeners of PCDDs and 135 congeners of PCDFs. The toxicity and biological properties of the different congeners probably have a common mode of action, the potency, however, is very varied. The most well-known and well-studied congener is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

TCDD is one of the most toxic man-made chemicals. It is persistent in the environment and accumulates in biological systems; several TCDD metabolites have been identified, however, there is no indications that more active metabolites are formed. On the contrary, the metabolites have been demonstrated to possess lower toxicity. The toxic effects of TCDD may be delayed and are markedly cumulative upon repeated exposure and, when the exposure period is prolonged, even decreasing daily doses of TCDD will eventually result in a certain degree of toxicity, which means that TCDD is an extremely hazardous chemical.

Chemical determination of TCDD in trace amounts was not possible until quite recently. With the advent of gas-chromatographic techniques, the limits of detection for TCDD in commercial products in the 1960s were of the order of 0.5 to 1 mg/kg, Later reaching ug/kg levels, but these methods were still insufficiently sensitive to detect the low but still biologically effective levels of TCDD in tissues or in the food chain. In the early 1970s, analytical methods were developed which at last made it possible to detect TCDD in the environment following heavy spraying of 2,4,5-T-contaminated by TCDD. These methods have recently been refined in a few laboratories so that TCDD concentrations as low as 10 ng/kg can now be detected in many environmental samples. In recent years a substantial numbers of the PCDDs and PCDFs have been synthesized that can be used as analytical standards. The application of combined gaschromatographic -masspectrometric techniques has now made possible the isomerspecific analysis of the PCDDs and PCDFs in environmental samples and tissues down to the ppt level. However, the methods are expensive, timeconsuming and fraught with difficulties for the inexperienced analyst.

11.2 Conditions and levels of exposure

11.2.1 Occupational exposure

During the production of 2,4,5-trichlorophenol and related products, sudden high dermal exposure may occur when "runaway" reactions in the production process lead to a sudden increase in the formation of TCDD. These chemical reactions are exothermic and may result in explosion of the reaction vessels unless special safety measures are instituted. Workers may either be sprayed immediately with TCDD or become contaminated during clean-up operations.

Exposure occurs primarily on contact. The environmental levels of TCDD involved in these accidents have not been measured but could have been high. This is suggested by the very high concentrations of TCDD found in the upper portion of the reaction vessel that released the toxic cloud at Seveso, Italy, and by the fact that waste materials from the production of trichlorophenol in Missouri, USA contained TCDD in a concentration of 300-365 mg/kg.

Complete decontamination of factory areas where explosions have occurred has proved difficult when all possibilities of exposure were not eliminated, additional cases of illness among workers have occurred However, successful decontaminations have been performed in several cases where PCB-fire have caused heavy contamination with PCDFs.

In the past, workers have also been exposed to TCDD during the routine production of 2,4,5-trichlorophenol and related products (section 9.1, table 9-1). Exposure was again to a large extent dermal and some ingestion and inhalation of TCDD-contaminated dust probably also occurred, but qantitative information is not available. Some workers may have developed chloracne and systemic illness following continuous exposure to products containing TCDD at levels ranging from 10 to 25 mg/kg 2,4,5,-T (BLeiberg et al 1964; Poland et al 1971). In all, some 800 persons now living have at some time in the past been exposed to TCDD and developed chloracne or other illness (Reggiani 1977).

11.2.2 Exposure of the general population

Owing to the accidental release of TCDD from a factory at Meda near Seveso, Italy and a result of the spreading of industrial waste material on riding arenas in Missouri, USA, illness has occurred in the general population of those areas (section 9.2). At Seveso, so far, illness has been mainly confined to skin disease while in Missouri a few cases of systemic illness were also reported.

At the moment there is insufficient information on the levels of exposure in the recognized instances of environmental contamination by TCDD to draw conclusions on dose-response relationships.

In many parts of the world there may be very low environmental levels of PCDDs and PCDFs that have not yet been determined. This was first suggested by the TCDD that was found in some beef-fat samples collected in the USA (section 6.4). Recently, the occurrence of several PCDDs and PCDFs have been reported in various aquatic organisms including fish and most recently also in human fat and mothers milk from humans with no known specified exposure. Whether this contamination is limited to certain areas or whether it is more extensive is not yet known. TCDD and probably also other PCDDs and PCDFs are slowly degraded in the soil, and they thus enter the food chain and are probably biomagnified, but at the moment there is not enough evidence for assuming that this is so on a world-wide basis.

In general the level of air, water and soil contamination caused by TCDD is unknown except as regards some local high levels of soil contamination, (areas of Vietnam, sites in Florida and Missouri, USA; Seveso, Italy).

Whether PCDDs and PCDFs from other sources could contribute appreciable to any possible contamination of the environment is not known. It is however recognized that the emission from waste incineration plants contain appreciable amounts of PCDDs and PCDFs. Waste incineration has been calculated to contaminate the environment with several kg/year of the sum of PCDDs and PCDFs in e.g. Holland and Denmark. Milk from cows grazing nearby waste incinerators in Switzerland has been demonstrated to contain about 1 ppt (ng/kg) TCDD on a fat basis.

11.3 Metabolism

11.3.1 Animal data - TCDD

Studies on rats given a single oral dose, or repeated oral doses, of ^{14}C-TCDD have shown that more than 70% of the amount administered is absorbed from the gastrointestinal tract. Whole body half life times in the order of 22-42 days in the Guinea pig, 23-31 days in the rat and 10-12 days in the hamster have been reported. Several metabolites of TCDD have so far been identified (Sawakata et al 1982).

TCDD does accumulate in rat tissues, particularly in the liver and adipose tissue. The results of one study, in which ^{3}H-TCDD was given to rats and monkeys at the same oral dose/kg body weight, indicate a substantial interspecies difference in TCOD levels in different organs.

At a daily dose of 0.001 ug/TCDO/kg body weight for 2 years, rats accumulated 540 ng/TCDD/kg body weight (ppt) in the liver where some morphological changes were also observed. Similar TCDD levels were found in beach mice that had been exposed to TCDD soil levels ranging from 10-710 ng/kg indicating that extensive total body exposure to TCDD in soil at such (ppt) concentrations in animals may still result in TCDD tissue levels sufficient to cause some effects.

TCDD is largely eliminated with the faeces; some urinary excretion of ^{14}C or ^{3}H-labelled material also occurs in rats or monkeys given ^{14}C or ^{3}H-labelled TCDD. The hamster has a higher urinary elimination than other species studied.

11.3.2 Animal data other PCDDs and PCDFs

Animal data on the toxicokinetics of other PCDDs than TCDD seems to be lacking. PCDFs have been more studied in this respect. The half life for 2,3,7,8-TCDF has been reported to less than 2, 2, 4, more than 20 and 8 days for rats (Fischer 344), mice (C57B1/6J), mice (DBA/2J), guinea pig and Rhesus monkey respectively (see table 10-1). Other studies have on rats have shown that 2,3,4,7,8-PeCDF was more highly retained than was 2,3,7,8-TCDF (65% and 3,8% respectively after 5 days).
However, some of these data have been collected from studies where the exposed animals have been given dosages that high that they caused a high degree of enzyme induction and also overt signs of toxicity. The data are thus not easy to interpret.

11.3.3 Human data - TCDD

Recently, TCDD levels were measured in a woman who had been exposed to TCDD in her home environment in an area where soil contamination ranged from 162-1847 ug/m^2. The highest concentration (1.84 ug/kg) was found in the adipose tissue; in the pancreas the TCDD concentration was 1.04 ug/kg and in the liver 0.15 ug/kg.

In human fat levels of TCDD at 2-3 ppt (ng/kg) have been found in human with no known specified exposition. Human mothers milk have been found to contain TCDD at the level 1-2 ppt calculated on fat basis (Table 10:2).

11.3.4 Human data - other PCDDs and PCDFs

No systematic toxicokinetic data on the elimination in man exist. However, from the analysis performed both on human fat and human mothers milk it is obvious that only PCDDs and PCDFs containing 4 chlorine atoms in symmetrical lateral position (i.e. 2,3,7,8) are retained at measurable levels. OCDD, OCDF, TCDD and TCDF, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF, 3 isomers of HxCDDs and 3 isomers of HxCDFs and 1,2,3,4,6,7,8-Hepta-CDD and CDF has thus been identified and quantified in human mothers milk.

11.4 Toxic effects in animals

11.4.1 TCDD

TCDD is extremely toxic to all animal species tested. The oral LD_{50} is in the microgram/kg range. The guinea-pig has so far proved to be the most sensitive species as regards acute toxic effects, and the oral LD_{50} is about 0.6 to 2.1 ug/kg body weight. Acute toxicity in animals following dermal exposure has not been extensively studied but lethal effects have been observed in rabbits following the application of TCDD to the abdominal skin, (the animals being prevented from ingesting this TCDD). No inhalation studies have so far been reported.

A multitude of toxic effects were observed in animals following a single dose of TCDD, or long-term exposure, but not all of these effects were found in all the species under study. The animals, of all the species treated, suffered severe weight loss after single or repeated toxic doses and death was delayed up to 7 weeks after a single lethal dose. Effects on the thymus were found in all species given lethal or even sublethal doses of TCDD. Severe liver damage following a lethal dose of TCDD occurred in rats, rabbits and -to a certain extent - in mice but not in guinea-pigs. Effects on the bone marrow, anaemia, leukopenia, thrombocytopenia and haemorrhage also occurred in several species including monkeys. Skin lesions similar to those observed in humans were found in rabbits, particularly on the rabbit ear, following dermal or even intra-peritoneal exposure, and conjunctivitis, enlargement and squamous metaplasia of the Meibomian glands with keratosis have been observed in monkeys. Other effects are chick oedema disease, effects on the vascular system of the horses involved in the accident in Eastern Missouri, and increased mesenteric peri-arteritis in long-term experiments on rats.

Squamous metaplasia of some glandular epithelium and proliferation and dilations of the glandular crypts of the lining of the stomach have also been noted, particularly in sub-human primates. Toxic effects in sub-human primates have been produced at very low (500 ng/kg diet) concentrations in food. These dietary levels caused the death of 5 out of 8 monkeys within 7 to 12 months.

TCDD has demonstrated teratogenic potential in mice through mostly inducing cleft palate and kidney anomalies. At higher doses fetotoxic effects was demonstrated.
In rats TCDD seems to be more fetotoxic to rat in comparison to mice through demonstrating decreased fetal weight, intestinal hemorrhage and edema. Malformation predominantly in form of kidney-malformations has been noted in doses above 0.5 µg/kg. A NOEL or possibly a LOEL of 0.001 µg/kg bw and day for reproduction in rats have been discussed (Murray et al 1979, Nisbeth and Pascton 1982).
In rabbits and monkeys fetotoxic effects of TCDD is reported. The studies performed are too limited to establish a teratogenic response.

Sections giving more details on these and other effects will be completed after the discussion of the Task Group on section 8.

A study involving a targe number of rats subjected to dietary TCDD exposure (daily doses of 0.1 ug TCDD/kg body weight for 2 years) resulted in hepatocellular carcinomas in the female and squamous cell carcinomas of the lungs, nasal turbinates, hard palate and tongue of males and females. Doses of 0.01 ug/kg body weight caused an increased incidence of neoplastic (hyperplastic) nodules in females, and doses of 0.001 ug/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 TCDO. Thus TCDD has been shown to be carcinogenic, particularly in rats, at dietary levels that are much lower than for any other carcinogen so far tested.

In a study with smaller numbers of rats, that were given dietary levels of 5, 50 or 500 ng TCDD/kg feed, a variety of individual tumours were noted. At the 5 ug/kg feed level, an increased incidence of liver and lung tumours was found, but it is difficult to evaluated this study because of the small number of animals.

A study in Swiss H/Riop mice, with combinations of TCDD and 2,4,5-trichlorophenoxyethanol, also resulted in an increase of liver tumours, thus supporting the finding of carcinogenicity recorded in rats.

11.4.2 Other PCDDs and PCDFs

Several other PCDDs and PCDFs cause signs and symptoms similar to those of TCDD but there is a wide variation with regard to potency. In summary, there are 12 isomers that displays high toxicity, i.e. the tetra-, penta-, hexa- and hepta-CDDs 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-HCDD) has been demonstrated to possess carcinogenic properties in long term animal studies but higher doses than those of TCDD. Unsubstituted dioxin and 2,7-dichlorodibenzo-p-dioxin failed to demonstrate carcinogenic properties.

11.4.3 Review of species differences

One of the interesting features of TCDD toxicity is the pronounced species difference in sensitivity (see table 8-1). Oral LD_{50} varies from 0.6 yg/kg b w in guinea pigs to 5051 yg/kg b w in Syrian Golden hamster. Also with different strains within the same species, pronounced differences occur, e.g. oral LD_{50} for Sherman rats in the range 13-43 yg/kg b w while oral LD_{50} in Fischer 334 N in the range 164-340 yg/kg b w.

The tremendous variation in species sensitivity to TCDD and related compounds cannot be explained by differences in metabolic rate, clearance times, body burden of the compounds, or by macromolecular adduct formation.

Differences in the toxic response to TCDO have been found (see table 8-2) but the mechanism underlying these differences is still not clarified. One proposed model for the mechanism of action of TCDO and related compounds is based on the interaction with the cytosolic Ah-receptor(s). However, the binding affinity and concentrations of hepatic cytosolic receptor are similar for guinea pig, rat, C57B mice, rabbit and hamster, yet there exists a 5 000-fold difference in LD_{50} values for TCDD between guinea pig and hamster. Furthermore, the correlations that exist between cytosolic receptor concentrations and toxicity in inbred strains of mice are not present in other species.

11.5 Human health effects

11.5.1 TCDD

Several follow-up studies have been performed on exposed workers (see section 9.3). Although some statistically significant differences can be found between exposed and controls there is a lack of uniformity between the different studies which might indicate that exposure to other compounds may have interferred. The study of Thiess et al (1982) reports a cancerincidence higher than expected and which cannot be explained as a mere chance event. However, the small size of the cohort and the small number of deaths from any particular case does not permit any definite conclusions concerning the carcinogenic effect of the exposure. The overall impression from the follow-up studies is

that even severe acute toxicological effects of TCDD are reversible or markedly improved over time. It thus seems unlikely that permanent, severe and debilitating toxicological sequelae are inevitable after exposure to TCDD sufficient to produce chloracne.

In Seveso, the only clear-cut adverse health effect recorded has been chloracne. 193 cases of chloracne occurred, 20 of those presented active symptoms still in 1984. 15 to 20 days of exposure to soil contaminated with 370-1200 $\mu g/m^2$ clearly caused chloracne while exposure to soil levels at or below 30-70 $\mu g/m^2$ did not cause measurable effects.

During the period 1960-69 a mixture of 2,4-dichloro- and 2,4,5-trichloro-phenoxyacetic acid contaminated with TCDD (0.5-47 ppm) was heavily used in Vietnam as a defoliant. Many studies have been performed to find possible links between exposure and health effects on people exposed in Vietnam or soldiers engaged in the spraying. However, due to many causes, the material available to date does not allow any conclusions to be drawn with regard to effects on human reproduction or any other significant health effects (see section 9.2).

11.5.2 PCDFs

The only well documented intoxications with PCDFs in humans are the two cases of contamination of rice oil with PCBs and related hydrocarbons, i.e. Yusho in Japan 1968 and Yu-cheng in Taiwan 1979 (see section 9.4). In total several thousands of people were intoxicated. The summarized data makes it most likely that the causative agent was the PCDFs occurring as contaminant in the PCBs that had contaminated the rice oils. With minor differences, the symptomatology is quite similar to what is seen in intoxications with TCDD. Attempts to estimate the daily intake of PCDFs in Yusho patients indicate a figure of 0.9 yg/kg b w together with 157 yg PCBs and 148 yg PCQs/kg b w (Hayabuchi et al 1979). However, the data available are not enough to allow any conclusions as to what dose might by safe for human intake.

11.6 Risk evaluation

All available information from animal studies demonstrates the extreme toxicity of TCDD. There are however, considerable species differences. In rodents, cancer and other toxic effects result from very low doses. No good quantitative information is available on the doses of TCDD that are toxic to man, but the symptoms and signs of toxicity exposure in man are similar. Follow-up studies of groups of men, occupationally or accidentally exposed, give the overall impression that man does not belong to the most sensitive species.

Despite all the studies conducted on animals, no definite information is available on the no-observed-effect levels in primates for long-term exposure. Moreover, the carcinogenic effects of TCDD observed in animal studies and the fact that certain morphological alterations were induced by feeding rats for 2 years levels as Low as 1 ng/kg body weight (the lowest exposure level used) would suggest that long-term exposure, even to concentrations below or at the detection limits of the analytical procedures presently available could present a certain risk to the population.

Several risk evaluations on TCDD have been performed in various countries. Some of them have used the long term oncogenic fat study as the basis (Kimbrough et al 1978). Using these data various extrapolations have been made to determine a "virtually safe dose", i.e. a dose that would given one extra cancer case in 10^6 humans and year (Kociba et al 1984, EPA 1984). Other risk evaluations have used the no-observed-effect level or the lowest-effect level from long term or reproduction studies and applied a "safety factor" in the range 200-1 000 (e.g. Denmark 1984).

The studies on the mutagenicity and carcinogenicity indicate that TCDD is not a genotoxic compound. A few reports have given some evidence of such an effect but these are not convincing when compared to the reports stating the contrary. On the other hand TCDD has been strongly positive as a promoter in two-stage precarcinogenesis study (Pitot et al 1980). It has been argued that the straight forward matematical extrapolation from animal studies thus should not be used. One risk evaluation (Denmark 1984) accordingly has used the no-effect level in the long term cancer study on rats (Kociba et al 1978) and

applied a safety factor of 200 while other use varying extrapolation from the same study and determines a "virtually safe dose" at specified conditions (Kimbrough et al 1984).
Regardless of the approach used, these evaluations have ended up with a suggested "tolerable daily intake" for humans in the range 1-5 picogram per kg bodyweight.

The recently available analytical data on TCDD in human milk indicates level at or slightly above 1 ppt on a fat basis. Assuming a daily intake of 850 ml milk in a baby with a bodyweight of 5 kg this would result in a daily intake of TCDD in the range 5-10 pg/kg depending on the fat level of the milk. This calculation would indicate that the environmental levels of TCDD alone are such that, at least in industrialized countries, the tolerable daily intake would be approached or exceeded.

Analysis however, have also demonstrated the occurrence of several other PCDDs and PCDFs in the environment. There are some reasons to believe that these may act additive in effect to TCDD. It is thus necessary to evaluate the risk for these congeners when they appear together. Several attempts of such estimates have been tried. The approaches used have been to relate the toxicity of various PCDDs and PCDFs to that of TCDD and express the toxicity in "TCDD-equivalents". These approaches have been summarized by Bellin and Barnes (1984) and table 10-1 gives the conversion factors for some of these approaches.

Table 10-1: Some approaches to estimating relative toxicities of PCDDs and PCDFs. Modified from Bellin and Barnes (1984).

	Method					
Compound	1	2	3	4	5	6
Cl 1-2	-	-	-	-	-	-
Cl 3	-	-	-	1	-	0.001
2,3,7,8-TCDD	1	1	1	1	1	1
other TCDOs	0.01	-	-	0.01	0.01	0.01
2,3,7,8-PeCDDs	0.1	0.1	1	1	0.2	0.1
other PeCDDs	0.1	-	-	0.01	0.002	0.1
2,3,7,8-HxCDDs	0.1	0.1	0.3	1	0.04	0.1
other HxCDDs	0.1	-	-	0.01	0.004	0.1
2,3,7,8-HpCDDs	0.01	0.1	-	1	-	0.01
other HpCDDs	0.01	-	-	0.01	-	0.01
OCDD	-	-	-	1C?)	-	
2,3,7,8-TCDF	0.1	0.1	0.33	0.02	0.1	0.1
other TCDFs	0.1	-	-	0.0002	0.001	0.1
2,3,7,8-PeCDFs	0.1	0.1	0.33	0.02	0.1	0.2
other PeCDFs	0.1	-	-	0.0002	0.001	0.2
2,3,7,8-HxCDFs	0.1	0.1	0.01	0.02	0.1	0.1
other HxCDFs	0.1	-	-	0.0002	0.001	0.1
HpCDFs	-	-	-	0.02C?)	-	0.01
OCDF				0.02(?)		-

Method 1 Switzerland 1982
2 Grant et al 1977, Olieet et al 1983, Commoner 1983
3 Eadon et al 1982
4 Ontario government 1982
5 USEPA 1984
6 Denmark 1984

The procedures described are in the following applied to a human mothers milk sample that have been analyzed with isomer specific GC-MS analysis.

Table 11-2. Levels of PCDDs and PCOFs in human mothers milk and TCDD-equivalents using different methods (analytical data from Rappe, 1984).

Isomer	Level pg/g fat	Method 1	2	3	4	5	6
PCDDS							
2378-TCDD	1	1	1	1	1	1	1
2378-PeCDDs	3.6	0.36	0.36	3.6	3.6	0.72	0.36
2378-HxCDDs	24	2.4	2.4	7.2	24	0.96	2.4
2378-HpCDDs	38	0.38	3.8	0	38	0	0.38
OCDD	225	0	0	0	0	0	0
PCDFs							
2378-TCDF	3.6	0.36	0.36	1.188	0.072	0.36	0.36
2378-TCDFs	11	1.1	1.1	3.63	0.22	1.1	2.2
2378-HxCDFs	6.1	0.61	0.61	0.061	0.122	0.61	0.61
HpCDFs	4.4	0	0	0	0.088	0	0.044
OCDF	11	0	0	0	0	0	0
SUM **TCDD-EQIUV**		6.21	9.63	16.68	67.10	4.75	7.35
FAT LEVEL X:	2.2						
LEVELS IN MILK: **pg/l**		136.62	211.86	366.94	1476.24	104.5	161.79
PG/KG B W AND DAY FOR A	5 KG BABY	23.23	36.02	62.38	250.96	17.77	27.50

Note: No conversion factor used for OCDD and OCDF in method 4 as we do not understand the scientific basis for these values A daily milk consumption of 850 ml is assumed.

Methods: 1. Switzerland 1982.
2. Grant et al 1977, Olieet et al 1983, Commoner et al 1983.
3. Eadon et al 1982.
4. Ontario government 1982.
5. USEPA 1984.
6. Denmark 1984.

From table 11:2 it is apparent that applying the different methods to a sample of Swedish human milk indicates that the daily intake of "TCDD-equivalents" with marginal overrides the calculated "tolerable daily intake" (1-5 pg/kg bw) for TCDD as such.

It is recognized that an approach such as using "TCDD-equivalents" has its value as an interim procedure for evaluating the toxicity of environmental samples. However, it is apparent that such an evaluation contains a high degree of uncertainty.

It is interesting though to note that with the exception of method 4, there seems to be a fairly good agreement between the values arrived at with the different methods although the basis for some of them are quite different i.e. method 1 and 6 use enzyme induction as a basis, method 3 uses acute toxicity and method 5 is based on extrapolation from cancer studies.

The approach has two levels of uncertainty, i.e. firstly there is an uncertainty in the evaluation of TCDD by itself, secondly in the fractional value given the different congeners.

11.6.1 Problems in the evaluation of TCDD-risk

The pronounced species differences in toxicity makes the extrapolation to man more uncertain than usual. Knowing the mechanism of action of TCOD would probably give a better foundation for the evaluation. Several theories concerning the mechanism of action have been proposed. However, none of these has been able to explain the species differences in an acceptable way.

The induction of drug metabolizing enzyme system has been suggested as one major mechanism of action. The induction is believed to be mediated through a cytosolic receptorprotein. The results of several studies of different congeners indicate that the binding affinity and the potency to induce microsomal monooxygenases correlate fairly well with the toxicity in vivo. However, neither the levels of the receptor and nor the differences with regard to enzyme induction can explain the large species variation in sensitivity. The amount of cytosolic receptor protein is thus about the same in the liver of guinea pigs, rats, mice, rabbits and hamsters although the acute toxicity varies with a factor of more than 1 000. Furthermore, the LD_{50}-dose of 2,3,7,8-TCDD in chicken embryos, C3H/HeN-mice and SD-rats varies more than 100-fold although the dose that causes half maximal induction of AAH is about the same. It has been suggested that the

receptor is a necessary but not the only factor determing the toxicity of the compounds. The toxicity thus depends on a continuing onset of a endogenous cellular regulating system, the nature of which is not known.

Several other reported effects of TCDD on vital physiological mechanism may as well be of importance in explaining the toxicity and also the species variation in sensitivity. Such areas are interactions with the vitamin A turnover (H§kansson och Ahlborg 1985, Thunberg et al 1979, 1980, Thunberg och Håkansson 1983) effects on the function of plasmamembranes and their build up (Brewster et al 1982, Matsumura et al 1984) and effects on the formation of keratin and celldifferentiation (Gierthy et al 198A, Knutson and Poland 1980, 1982, Puhvel et al 1984, Rice and Cline 1984).
All these physiological processes are characterized by being equilibrium processes that occur in all the cells in the organism. Depending on which c ellsystem, the balance may be in a different state. There are in this regard also differences between both species and sex. A better understanding of these processes may thus be expected to enlighten our understanding of the varying effects of PCDDs and PCDFs on different tissues and species.

11.6.2 Risk groups

The calculated "tolerable daily intake" of 1-5 picogram/kg body weight assumes life-long exposure. However, no special precautions have been made with regard to the risks for infants. In general, infants are regarded as being more sensitive to toxic chemicals since their mechanisms of detoxification *are* not fully developed.

One of the effects of PCDDs and PCDFs further indicates that the newborn may be at higher risk. The compounds interfere with the organism ability to utilize vitamin A. The child is born with low levels of vitamin A in the body. The stores are built up during the neonatal period (Gebre-Medhin and Vahlquist 1984). The hepatic levels of vitamin A in newborns with good nutritional status (100-300 Y9 vitamin A/g) are in the same range as those found in the rat (Flores et al 1984). At the moment there is no easy way to determine the vitamin A status in the infant. The measurement of vitamin A in blood does not reflect the whole body status. The only ways are to either measure the hepatic concentration which is only possible in special circumstances or estimate the consumed amounts

Table 4-12. Levels of PCOFs in samples from **PCB** fires (From Rappe, 1984)

	ΣCl_4	2378-	ΣCl_5	ΣCl_6	ΣCl_7	ΣCl_8	ΣPCDDs
Binghamton (yg/g	20	12	670	965	460	40	20
Stockholm ($\mu g/m^2$)	1.2	0.15	0.175	-	-	-	-
Skövde ($\mu g/m^2$)	0.6	0.1	0.1	0.06	0.008	0.006	-
Surahammar ($\mu g/m^2$) (before cleaning)	1.2	0.3	0.3	0.15	0.07	0.01	-
Surahammar ($\mu g/m^2$) (after cleaning)	< 0.02	< 0.004	< 0.01	< 0.01	< 0.015	< 0.002	-
Hallstahammar ($\mu g/m^2$) (before cleaning)	1.6	0.54	0.36	0.60	0.80	1.34	-
Hallstahammar ($\mu g/m^2$) (after cleaning)	0.002	0.0002	0.0005	0.0003	0.0007	0.0011	-
Electrical locomotive ($\mu g/m^2$)	4.9	1.2	1.5	0.4	0.17	0.04	-

Table 5-4. Levels of TCDD's in Fish and Shellfish (Mitchum et al 1980)

Type/Section of Fish	Sampling Site	Type of TCDD	Concentration ng/kg
edible flesh	Bayou Meto/ Arkansas River	2,3,7,8-TCDD	480
catfish	-"-	-"-	ND (7 ng/kg)a-50
buffalo	-"-	-"-	ND (7-13 ng/kg)[a]
bottom feeder	-"-	-"-	77
turtle / egg and liver	-"-	-"-	15
snake / like and muscle	-"-	-"-	60
muskrat / liver	-"-	-"-	ND (40 ng/kg)[b]
racoon / liver	-"-	-"-	ND (10 ng/kg)b
frog / liver and muscle	-"-	-"-	> 10

a These are averages of samples that had above detectable Levels of TCDD.

b Not reported and the limit of detection indicated in parentheses.

NR Not reported.

Table 5-7. **Levels** of PCDDs and PCDFs in samples from the Baltic Sea (ng/kg), (Rappe et **al** 1984)

Isomer	Herring Utlängan	Herring W. Gotland	Guillemot Karlsö
2,3,7,8-Tetra-CDF	4	3	2
2,3,6,7- -"-	1	1	T
1,3,4,8,9-Penta-CDF	-	-	T
1,2,4,6,8- -"-	T	T	
1,3,4,7,9-/1,2,3,6,8- -"-	T	T	T
1,2,4,7,8- -"-	1	1	
1,2,3,7,8-/1,2,3,4,8- -"-	1	1	4
2,3,4,7,8- -"-	6	6	180
1,2,3,4,6,8-Hexa-CDF	T	T	
1,3,4,6,7,8- -"-	T	T	T
1,2,3,4,7,8-/1,2,3,4,7,9- -"-	1	1	9
1,2,3,6,7,8- -"-	T	T	25
1,2,4,6,8,9- -"-	T	T	-
2,3,4,6,7,8- -"-	1	1	7
2,3,7,8-Tetra-CDD	-	-	7
1,2,3,7,8-Penta-CDD	-	T	22
1,2,3,4,7,8-Hexa-COD	-	-	16
$^{13}C_{12}$-Tetra-CDF (recovery)	88%	78%	79%
$^{13}C_{12}$-Tetra-CDD (-"-)	71%	78%	67%
$^{13}C_{12}$-Octa-CDD (-"-)	44%	33%	A9%

T Traces; **identified** but normally less than 1 ppt.

Table 5-10. Analytical results for 2,3,7,8-TCDD residues (Harless et al 1983)

Sections of Eleven Deear in study	No. of Deer samples analyzed	No. of positive samples	Concentration Range of 2,3,7,8-TCDD detected (ng/kg)[a]	Limit of detection range (ng/kg)[a]
Muscle	11	3	12 to 27	0.5 to 5
Adipose Tissue	10	8	3 to 12	1 to 3
Liver	11	4	2 to 5	0.4 to 4
Bone Marrow	5	0	ND	1 to 3

[a] Results corrected for sample preparation efficiency.

NO - not detected

Table 5-11. Tissue concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin. (Facchetti et al 1980)

Sample[a]	2,3,7,8-TCDD/wet tissue ng/kg
Fat	1840[b]
Pancreas	1040[b,c]
Liver	150[b,c]
Thyroid	85[c]
Brain	60[b]
Lung	60[b]
Kidney	40[b]
Blood	6[c]

[a] The investigation of other available samples is being completed.

[b] Values obtained at a resolution of 2 500.

[c] Values obtained at a resolution of 10 000.

Table 7-1. Average pharmacokinetic parameter estimates for the elimination of ^{14}C-activity from male and female rats administered 0.1 or 1.0 yg of ^{14}C-TCDD/kg/day, monday through friday, for 7 weeks[a] (Rose et al. 1976).

	k (days^{-1})[b]		fc	
Rat sex	1.0 yg/kg/day	0.1 µg/kg/day	1.0 yg/kg/day	0.1 yg/kg/day
Male	0.256±0.0050	0.028610.0213	0.733±0.104	0.87010.061
Female	0.0356±0.0031	0.027310.0021	0.931±0.023	0.910±0.013

[a] Each parameter is expressed as the mean ± SD of 3 rats. No statistically significant (Mann-Whitney) differences due to sex or dose level.

[b] Elimination rate constant.

[c] The fraction absorbed.

Table 7-2. The level of ^{14}C-activity in selected tissues expressed as µg equivalents of TCDD per kg of tissue for rats given ^{14}C-TCDD at doses of 0.01, 0.1 or 1.0 µg/kg body weight/day, monday through friday, for 1, 3 or 7 weeks[a] (Rose et al. 1976)

Exposure for:	1 week	3 weeks	7 weeks
	Exposure level 1.0 µg/kg/day		
liver	49.5+3.6	110.2+37.1	204+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/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[b]	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 pg/kg/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] The mean ± SO of 3 male and 3 female rats

[b] Not detected

[c] Indicates the number of animals with detectable levels of ^{14}C-activity

Table 7-3. Distribution of radioactivity in Guinea pig tissue 1-15 days after administration of a single dose of 2.0 µg ^{14}C-TCDD/kg body weight (Gasiewicz and Neal 1979)

Tissue	^{14}C activity (% of dose/g tissue) at day					
	1	3	5	7	11	15
Perirenal adipose	3.2±1.0[a]	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	1.710.2
Liver	1.1+0.4	1.5+0.4	1.3+0.2	1.1+0.2	2.2±0.2	3.210.3
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	0.410.3	0.310.1
Stomach	0.2±0.1	0.3+0.1	0.1+0.1	0.2+0.1	0.310.1	0.310.1
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	0.810.4	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	6.510.7	6.7+0.6
Brain, heart, skeletal, muscle	<0.25					

[a] Values are means + SE for groups of three animals, unless indicated otherwise.

[b] Mean of two animals

[c] % of dose/g tissue

Table 7-4. Subcellular distribution of radioactivity in Guinea pig liver after a single dose of radiollabeled TCDD (Gasiewicz 1979).

Dose <yg TCDD/kg)	Fraction	Percentage of total radioactivity on day 1	6
7.0 (^{14}C)	Whole liver	0.7±0.1[a,b]	2.4±0.8[b]
	Crude nuclear	35.614.4	16.710.3
	Mitochondrial	9.510.2	9.911.0
	Microsomal	47.413.7	70.1±0.9
	Soluble	7.611.1	3.210.4
2.0 (^{14}C)	Whole liver	0.7±0.1[b]	-
	Crude nuclear	20.111.5	-
	Mitochondrial	12.9±2.5	-
	Microsomal	40.713.1	-
	Soluble	26.410.9	-
0.3 (^{3}H)	Whole liver	1.1±0.1[b]	2.0±0.2[b]
	Crude nuclear	31.3±5.1	28.611.6
	Mitochondrial	9.5±0.3	9.611.5
	Microsomal	44.8±6.2	49.212.6
	Soluble	14.3±0.6	12.610.7

[a] All values are the mean ± SE of three animals.
[b] Values are expressed as X of dose/g tissue.

Table 7-5. Experimental design and lethality of soil TCOD in guinea pigs: N.D., none detected (detection limit, 100 parts per trillion); S.E.M., standard error of the mean. The TCDD added to corn oil or soil was synthesized by the chemistry branch, National Institute of Environmental Health Sciences; purity, > 99 percent by gas chromatography. It was used to "spike" soil samples at a concentration of 0.8 µg per gram of soil (McConnell et al 1984).

Group	N	Source	Amount	TCDD (microgram per kilogram of body weight)	Dead/ treated	TCDD content of liver (ppb S.E.M.)
1	6	Corn oil	0.1 ml/100 g	0	0/6	N.D.
2	6	TCDD in corn oil	0.1 ml/100 g	1	1/6	1.6-2[a] 4.1[b]
3	6	TCDD in corn oil	0.1 ml/100 g	3	6/6	13.3
4	6	Times Beach soil	0.35 g	1.3	0/6	<1.0
5	5[c]	Times Beach soil	1.07 g	3.8	1/5	1.0±0.1[a] 3.2[b]
6	5[d]	Times Beach soil	3.60 g	12.8	5/5	34.3±6.0
7	6	Minker Stout soil	0.26 g	1.1	0/6	<1.0
8	6	Minker Stout soil	0.80 g	3.3	2/6	1.4±0.3[a] 2.0±0.1[b]
9	6	Minker Stout soil	2.67 g	11.0	6/6	25.7±5.2
10	5*	Times Beach soil, uncontaminated	3.60 g	0	0/5	N.D.
11	6	Times Beach soil, uncontaminated with TCDD added	2.71 g	10	6/6	45.4±8.4

[a] Animals that were killed at 30 days. [b] Animals that died before 30 days. [c] One animal died 2 days after dosing (not included). d One animal died at the time of dosing as a result of an error in the anethesia. [e] One animal died at the time of dosing as a result of an intubation error.

Table 7-6. Distribution of radioactivity in hamster tissues following a single oral dose of 650 µg ^{3}H-TCDD/kg body **weight** (Olson et **al.** 1980).

Tissue	Tissue content of ^{3}H (% of dose/g **tissue**)[a]			
	Day 1	Day 3	Day 10	Day 20
Liver	4.03±1.00	5.3210.82	3.19±0.93	0.86±0.09
Liver[b]	12.74±3.21	20.44±3.45	9.69±0.99	3.70±0.29
Peri renal adipose	2.93±0.87	3.4810.56	1.38+0.28	0.3210.03
Adrenals	1.56±0.52	1.1210.14	0.47±0.08	0.1010.01
Pancreas	0.39±0.20	0.6110.13	0.62±0.26	0.21±0.04
Kidneys	0.60±0.16	0.6410.11	0.60±0.32	0.1210.03
Spleen	0.30±0.08	0.24±0.05	0.43±.26	0.0710.02
Thymus	0.49±0.14	0.34±0.11	-	0.05±0.02
Skin	0.8410.26	0.31±0.07	0.56±0.18	0.0310.01
Stomach	0.3410.07	0.5510.09	0.6510.39	0.1610.06
Duodenum	0.5110.13	0.4710.09	0.55±0.28	0.07±0.02
Jejunum	0.5910.15	0.71±0.20	0.39±0.16	0.0810.02
Ileum	0.4110.12	0.3510.05	0.37±0.21	0.0610.02
Colon	0.9210.27	0.60±0.14	0.34±0.07	0.0610.01
Cecum	0.3910.11	0.4110.10	0.28±0.12	0.0510.01
Lungs	0.38±0.09	0.3710.05	0.41±0.25	0.0710.03
Skeletal muscle	0.2010.07	0.1510.05	0.1510.03	0.0410.02
Heart	0.1410.03	0.1310.02	0.15±0.08	0.03±0.01
Testes	0.1010.04	0.32±0.13	0.13±0.04	0.0310.01
Blood	0.12±0.02	0.14±0.03	0.12±0.06	0.0210.01
Brain	0.0310.01	0.0510.01	0.06±0.02	0.01

[a] All values are the mean 1 SE of four hamsters
[b] Percentage of dose/liver

Table 7-7. Transfer of TCOD to the mouse fetus following a single dose of ^{14}C-TCDD[a,b] (Nau and Bass 1981)

Tissue	^{14}C-activity in 5! maternal dose/g tissue (^{14}C-activity in ng TCDD/g tissue)								
	Dose (5 µg/kg)			Dose (12.5 µg/kg)			Dose (25 µg/kg)		
	oral	s.c.	i.p.	oral	!.C.	i.p.	oral	s.c.	i.p.
Maternal liver	6.5 (12.7)	4.1 (8.0)	5.2±2.1 (10.2*1.1)	6.3*1,1 (30.3±5.3)	4.3±2.1 (21±10.2)	5.0+0.95 (24*4.4)	5.7 (56)	8.8 (87)	10.5 (103)
Maternal lung	0.31 (0.61)	0.55±0.06 (1.1±0.12)	0.76*0.29 (1.5*0.6)	0.30*0.06 (1.5*0.3)	0.45 (2.2)	0.66+0.19 (3.2+0.92)	-C	-e	1.8
Maternal kidney	0.50 (0.98)	0.37 (0.73)	0.59±0.33 (1.2*0.65)	0.37±0.03 (1.8*0.14)	0.36±0.11 (1.7*0.53)	1.27±0.11 (6.2+0.53)	0.33 (3.2)	1.27 (12)	1.33 (11)
Placenta	0.18 (0.35)	0.46 (0.90)	0.62*0.03 (1.2*0.05)	0.15+0.06 (0.73±0.29)	0.42 (2.0)	1.80±0.58 (8.7+2.8)	0.27 (2.6)	1.69 (16)	0.89 (8.3)
Fetal liver	0.29 (0.57)	0.22 (0.53)	0.28 (0.54)	0.25 (1.2)	0.089 (0.43)	0.30 (1.5)	0.25 (2.4)	0.42 (4.1)	1.41 (14)
Non-hepatic fetal tissues[d]	0.083 (0.16)	0.080 (0.15)	0.14 (0.27)	0.083 (0.40)	0.045 (0.22)	0.095 (0.46)	0.061 (0.60)	0.087 (0.85)	0.12 (1.1)
Total fetal tissues[e]	0.10 (0.19)	0.080 (0.16)	0.15 (0.29)	0.10 (0.49)	0.058 (0.28)	0.12 (0.58)	-e	_e	-e

[a] Pregnant mice were given either 5, 12.5 or 25 µg (^{14}C)TCDD/kg body weight on day 16 of gestation either by the oral, s.c., or i.p. route. Two days later the animals were sacrificed and the various tissues analyzed as described in the Experimental section.

[b] Values are means * SD of groups of animals, or means of groups of 2 animals.

[c] Tissues not analyzed.

[d] Fetal livers were excised and the remaining fetal tissues were homogenized and analyzed.

[e] Total fetuses were homogenized and analyzed.

Table 8-1. Single lethal dose values for TCDD.

SPECIES/strain	Sex/No/group	Age/weight	Route/vehicle	Dose tested (µg/kg)	Duration of observation	LD_{50} (µg/kg)	Time to death (d)	Reference
GUINEA PIGS								
Hartley	M/NR	NR	oral/corn oil acetone (9:1)	NR	2-8 weeks	0.6	5-34	Schwetz et al 1973
Hartley	M/NR	NR	oral/corn oil acetone (9:1)	NR	2-8 weeks	2.1	9-42	Sehwetz et al 1973
Hartley	M/6	3-4 weeks/200-250 g	oral/corn oil	NR	30 days	2	17-20	McConnell et al 1978b
Hartley	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	Silkworth et »l 1982
Hartley	F/6	NR/500-600 g	oral/methyl-cellulose	0.1 0.5 2.5 12.5 20.0	42 days	19	12-42	Silkworth *t al 1982
RATS								
Porton	F/5-12	8-9 weeks/170-200 g	oral/DMSO	0 30 48 75 120 190 300	90 days	NR	40	Greig et al 1973
Porton	F/6	9-10 weeks/170-200 q	oral/arachis oil	0 126 199 315 500	90 days	NR	40	Greig et al 1973
Sherman	M/5-10	NR	oral/corn oil acetone (9:1)	8 16 32 63	2-8 weeks	22	9-27	Schwetz et al 1973
Sherman	F/NR	NR	oral/corn oil acetone (9:1)	NR	2-8 weeks	45	13-43	Sehwetz et al 1973
Sprague-Dawley	M/6	adult/NR	i.p./olive oil	4 doses 20-80	20	60	NR	Beatty et al 1978
Sprague-Dawley	F/6	adult/NR	i.p./olive oil	4 doses 10-60	20	24	NR	Beatty et al 1978

Sprague-Dawley	M/6	25 days/NR	i.p./olive oil	4 doses 5-50	20	25	NR	Beatty et al 1978
Fisher 334N	M/7	11-12 weeks/230-280 g	oral/corn oil	0 75 150 225 275 325 375	30 days	340[a] 303[b] 164[c]	28[a] 26[b] 25[c]	Walden and Shiller 1985
CD	M/7	10-11 weeks/350-370 g	oral/corn oil	0 75 150 225 275 325 375	30 days	297[c]	25[c]	Walden and Shiller 1985
MONKEYS								
Macaca Mulatta	F/3	juvenile/2.1-2.6 kg	oral/corn oil	0 70 350	47 days	<70	14-34	McConnell et al 1978a
RABBITS								
New Zealand	M,F/NR	NR	oral/corn oil acetone (9:1)	NR	2-8 weeks	115	6-39	Schwetz et al 1973
New Zealand	M,F/NR	NR	dermal/acetone	31.6 63 126 252 500	3 weeks	275	12-22	Schwetz et al 1973
New Zealand	M,F/5	NR	i.p./corn oil	31.6 63 126 252 500	4 weeks	NR	6-23	Schwetz et al 1973
MICE								
C57Bl/6	M/14	3 months/23.6-30.8 g	oral/corn oil acetone (6:1)	0 100 150 200	2 months	114	15-30	Vos et al 1974
C57Bl/6	M/NR	7-15 weeks/14-30 g	oral/arachis oil	NR	35 days	126	21±1.6	Jones and Greig 1975
C57Bl/6	M/8	9 weeks/21-25 g	oral/corn oil	NR	30 days	284	22-25	McConnell et al 1978b

C57Bl/10	M/5	42-121 days/NR	oral/arachis oil	85 107 135 170 213	45 days	146	22-38	Smith et al 1981
C57Bl/10	F/5	42-121 days/NR	oral/arachis oil	85 107 135 170 213 269 338 426 536	45 days	>450	22-38	Smith et al 1981
C57Bl/6J	M/NR	NR	i.p./olive oil	NR	30 days	132	NR	Gasiewicz et al 1983[d]
DBA/2J	M/NR	NR	i.p./olive oil	NR	30 days	620	NR	Gasiewicz et al 1983[d]
B6D2F$_1$/J	M/NR	NR	i.p./olive oil	NR	30 days	300	NR	Gasiewicz et al 1993[d]
HAMSTERS								
Golden Syrian	M/5-6	NR/50-80 g	i.p./olive oil	0 500 1000 2000 3000	50 days	>3000	1-5	Olson et al 1980b
Golden Syrian	F/5	NR/50-80 g	i.p./olive oil	0 500 1000 2000 3000	50 days	>3000	14-32	Olson et al 1980b
Golden Syrian	M/5	NR/50-80 g	oral/olive oil	500 1000 2000 3000	50 days	1157	2-47	Olson et al 1980b
Golden Syrian	M/6	NR/70-120 g	oral/corn oil acetone (9:1)	0 300 600 1000 3000 6000	55 days	5051	9-43	Henck et al 1981
DOGS								
Beagle	M/2	NR	oral/corn oil acetone (9:1)	300 3000	2-8 weeks	NA	9-15	Schwetz et al 1973
Beagle	F/2	NR	oral/corn oil acetone (9:1)	30 100	2-8 weeks	NA	all animals survived	Schwetz et al 1973
CHICKENS								
Leghorn	NR	4-6 weeks/NR	oral/NR	NR	NR	25-50	12-21	Greig et al 1973

M = male, F = female, NR = not reported, NA = not applicable, i.p. = intraperitoneal.

a - supplied by Harlan, b = supplied by Frederick, c = supplied by Charles River, d = based on unpublished studies by Gasiewicz et *l 1981.

Table 8-2. Toxic responses following exposure to 2,3,7,8-TCDD: Species differences[a].

	Monkey	Guinea Pig	Cow[b]	Rat	Mouse	Rabbit[b]	Chicken[b]	Hamster
Hyperplasia and/or metaplasia								
Gastric mucus	++[c]	0	+	0	0			0
Intestinal **mucosa**	+							++
Urinary tract	++	++	+4	o	0			
Bile duct and/or gall bladder	++	o	+		++			o
Lung: focal alveolar				++				
Skin	++	0	+[d]	0	o	++		o
Hypoplasia, atrophy or necrosis								
Thymus	+	+	+	+	+		+	+
Bone marrow	+	+			±		+	
Testicle	+	+		+	+		+	
Other								
Liver lesions	4	±		++	+	++	+	±
Porphyria	0	0		+	++		+	0
Edema	+	0		Q	+		++	+

[a] References: monkey (McConnell et al 1978b, Norback and Allen 1973, Allen et al 1977), guinea pig (McConnell et al 1978b, McConnell 1980, Moore et al 1979, Turner and Collins 1983), ccw (McConnell 1980), rat (McConnell 1980, Kociba et al 1978a, Kociba et al 1979), mouse (Schwetz et al 1973, McConnell et al 1978b, Vos et al 1973), rabbit (Kimmig and Schultz 1957, Schwetz et al 1973, Vos and Beems 1971), chicken (Schwetz et al 1973, Norback and Allen 1973, Allen and Lalich 1962, Vos and Koeman 1970), hamster (Olson et al 1980b, Henck et al 1981).

[b] Responses followed exposure to 2,3,7,8-TCDD or structurally related chlorinated aromatic hydrocarbons.

[c] Symbols: 0 = lesion not observed, + = lesion observed (number of "+" denote severity), ± = lesion observed to a very limited extent, blank = no evidence reported in litterature.

[d] Skin lesions in cattle are observed, but they differ from the skin lesions observed in other species.

Adapted from Poland and Knutson, 1982.

Table 8-3. Estimated single oral LD_{50}-values for PCDDs[a]

Chlorination of PCDDs	Guinea Pigs (µg/kg)[b]	Mice (µg/kg)[b]
2,8	>300 000	NR
2,3,7	29 444	>3 000
2,3,7,8	2	284
1,2,3,7,8	3	338
1,2,4,7,8	1 125	>5 000
1,2,3,4,7,8	73	825
1,2,3,6,7,8	70-100[c]	1 250
1,2,3,7,8,9	60-100[c]	>1 440
1,2,3,4,6,7,8	>600	NR

[a] Source: McConnell et al 1978b

[b] Spearman-Karber method

[c] Estimated range due to variability in replicates

NR - Not reported

Table 8-4. Studies on chronic exposure to TCDD in laboratory animals.

SPECIES/Strain	Sex/No/Group	Doses tested	Treatment schedule	Parameters monitored	References
RATS					
Sprague-Dawley	M/10	0 ppt 1 PPt 5 PPt 50 ppt 500 ppt 1 000 ppt 5 000 ppt 50 000 ppt 500 000 ppt 1000 000 ppt	continuous in diet for 65 weeks	survival	Van Miller and Allen 1977
Sprague-Dawley	M and F/10	0.001 yg/kg/day 0.01 yg/kg/day 0.1 yg/kg/day	continuous in diet for 2 years	extensive histopathology, hematology and clinical chemistry	Kociba et al 1978, 1979
MICE					
Swiss	M/38-44	0 yg/kg/week 0.007 yg/kg/week 0.7 yg/kg/week 7.0 yg/kg/week	by gavage weekly for 1 year	histopathology	Toth et al 1979
MONKEYS					
Macaca mulatta	F/8	500 ppt	continuous in the diet for 9 months	extensive histopathology hematology and clinical chemistry	Allen et al 1977

Table 8-5. Effects of TCDD on humoral mediated immunity responses in adult animals.

SPECIES/Strain	Sex/Age/Weight	TCDD-exposure Frequency/route/dose	Parameter measured	Response	Reference
GUINEA PIGS					
Hartley	F/NR/256 g	8 weekly oral doses of 0.008, 0.04, 0.2 or 1.0 µg/kg body weight	primary serum tetanus antitoxin level secondary serum tetanus antitoxin level	no effect decreased	Vos et al 1973
MICE					
Swiss-Webster	F/4-7 weeks/NR	Fed 10, 20, 50, 100 or 500 ppb continuously in the diet for 5 weeks	primary and secondary sRBC[a] antibody level, primary and secondary serum tetanus antitoxin level	decreased decreased	Hinsdill et al 1980
C57Bl/6J	M/6-8 weeks/NR	4 weekly i.p. doses of 0.1, 1 or 10 µg/kg body weight	anti-sRBC[a] plaque forming spleen cells anti-TNP-BA[c] plaque forming spleen cells	decreased decreased	Clark et al 1981
C57Bl/6J	M/6-8 weeks/NR	single i.p. dose of 1.2, 6 or 30 µg/kg body weight	anti-sRBC[a] plaque forming spleen cells anti-SIII[b] plaque forming spleen cells	decreased decreased	Vecchi et al 1980
C57Bl/6 C3H/HeN DBA/2 AKR B6D2F$_1$	M/8-10weeks/NR	single i.p. dose of 1.2, 6 or 30 µg/kg body weight	anti-sRBC[a] plaque forming spleen cells	decreased	Vecchi et al 1983

[a] sRBC = sheep red blood cell, [b] SIII = type III pneumoccal polysaccharide, [c] TNP-BA = trinitrophenylated Brucella abortus
NR = not reported.

Table 8-6. Effects of TCDD on humoral mediated immunity responses in maternally exposed animals.

SPECIES/Strain	Time of TCDD exposure	Route/dose	Parameter measured	Response	Reference
RATS					
Fisher-344 N	Prenatal day 18 and/or postnatal days 0, 7 and 14	oral/5 µg/kg body weight	primary and secondary BGG[a] antibody level	no effect	Faith and Moore 1977
Fisher/Wistar	Prenatal day 18 and/or postnatal days 0, 7 and 14	oral/5 µg/kg body weight	primary and secondary BGG[a] antibody level	no effect	Faith and Luster 1979
MICE					
Swiss-Webster	4 weeks before mating, throughout gestation and lactation	in the diet/ 1, 2.5, 5, 10 or 20 ppb	primary and secondary sRBC[b] antibody level	no effect	Thomas and Hinsdill
			primary anti sRBC[b] plaque forming spleen cells	decreased	

a = BGG - bovine γ-globuline, b = sRBC - sheep red blood cell

Table 8-7. Effects of TCDD on cell-mediated immunity responses in adult animals.

SPECteS/Stratn	Sex/Age/Weight	TCDD-exposure frequency/route/dose	Parameter measured	Response	Reference
GUINEA PIGS					
Hartley	F/NR/256 g	8 weekly oral doses of 0.008, 0.04, 0.2 or 1.0 µg/kg body weight	delayed hypersensitivity to tuberculin	decreased	Vos et al 1973
RATS					
CD	F/NR/185 g	6 werkly er»l doses of 0.2, 1.0 or 5.0 µg/kg tofldyweight	delayed hypersensitivity to tuberculin	no effect	vos et at 1973
RABBITS					
New Zealand	M/NR/NR	2, 4 or 8 weekly doses of 0.01, 0.1, 1.0 or 10.0 µg/kg body weight	lymphoproliferative response of PHA[b]- and PWM[c]-stimulated spleen cells	decreased	Sharma et al 1978
			delayed hypersensitivity to tuberculin	decreased	
MICE					
C57Bl/6	M/6-8 weeks/NR	& weekly i.p. doses of 0.1, 1.0 or 10.0 µg/kg body weight	delayed hypersensitivity to sRBC[a]	decreased	Clark et at 1981
			delayed hypersensitivity to oxazalone	decreased	
			generation of alloantigenspecific cytotoxic T-cells	decreased	
C57Bl/6	M/NR/NR	4 weekly i.p. doses of 0.001, 0.01, 1.0 or 10.0	resistance to Herpes virus challenge	decreased	Clark et al 19(3
			generation of alloantigenspecific cytotoxic T-cells	decreased	
DBA/2	M/NR/NR	4 weekly i.p. doses of 0.001, 0.01, 0.1, 1.0 or 10.0 µg/kg body weight	generation of alloantigenspecific cytotoxic T-cells	decreased	Clark et «l 1983
Swiss-Webster	F/4-7 weeks/NR	5 weeks feeding of diets containing 10, 50 or 100 ppb TCDD	resistance to Salmonella typhimurium challenge	decreased	Hinsill et al 1980
			resistance to Listeria monocytogenes challenge	decreased	
			contact sensitivity to 2,4-dinitro-1-fluoro-benzene	decreased	
C57Bl/6J	M/6-8 weeks/NR	single i.p. doses of 1.2, 6 or 30 µg/kg body weight	number of peritoneal macrophages	decreased	Mantovani et al 1980
			number of splenic natural killer cells	decreased	
			macrophagemediated cytolysis	no effect	
			macrophage mediated cytostasis	no effect	
C57Bl/6 OB* $B6D2F_1$	M/NR/NR	4 weekly i.p. doses of 0.001 µg/kg body weight	generation of allospecific cytotoxic T-cells	decreased	Nagarkatti et al 1984
				no effect	
				decreased	
CD-1	M/NR/NR	2, 4 or 8 weekly doses of 0.01, 0.1, 1.0 or 10.0 ug/kg body weight	lymphoproliferative response of PHA[b]- and PWM[c]-stimulated spleenic cells	decreased	Sharma et al 1978
			delayed hypersensitivity to tuberculin	decreased	
C57Bl/6Jfh	KM weeks/NR	4 weekly oral doses of 0.S, 1.0, 5.0, 10.0 or 20.0 µg/kg body weight	resistance to Salmonella bern challenge	decreased	Thigpen et al 1975
			resistance to Herpes virus challenge	no effect	
C57Bl/6	M/2 months/ 24.4g	4 weekly oral doses of 0.2, 1.0, 5.0 or 25.0 ug/kg body weight	graft versus host activity	decreased	Vos et al 1973
C57Bl/6Sch	M/1 month/NR	4 weekly oral doses of 1.0, S.0 or 25.0 µg/kg body weight	lymphoproliferative response of PHA[b]-stimulated spleenic cells	decreased	Vos and Moore 1974
			graft versus host activity	no effect	
C57Bl/6Sch	M/4 months/NR	6 weekly oral do«e« of 1.0, 5.0 or 25.0 µg/kg body weight	lymphoproliferative response PHA-stimulated spleenic cells	no effect	vos and Moore 1974
			graft versus host activity	np effect	
Swiss	M/3-4 weeks/NR	4-5 weekly oral doses of 50 pg/kg body weight	resistance of Listeria monocytogenes	no effect	Vos et at 1978a
			number of peritoneal macropahges	no effect	
			macrophage reduction of nitroblue tetrazodium	no effect	

[a] sRBC . sheep red blood cells, [b] PHA . phytohemagglutinin, [c] PWM » poke weed, NR t not reported

Table 8-8. Effects of TCDD on cell-mediated immunity responses in maternally exposed animals.

SPECIES/Strain	TCDD-exposure Frequency/route/dose	Age when tested	Parameter measured	Response	Reference
RMS					
Fisher/Wistar	5 µg/kg body weight on gestationday 18 and on postnatal days 0, 7 and 14 or 5 ug/kg body weight on postnatal days 0, 7 and 14	25 days	lymphoproliferative response of PHA[a]- and ConA[b]-stimulated spleen and thymus cells	decreased	Faith and Luster 1979
			delayed hypersensitivity to tuberculin	decreased	
Fisher-344	5 µg/kg body weight on gestationday 18 and on postnatal days 0, 7 and 14 or 5 pg/kg body weight postnatal days Q, 7 and 14	25 days	lymphoproliferative response of PHA[a]- and ConA[b]-stimulated spleen and thymus cells	decreased	Faith and Moore 1977
			delayed hypersensitivity to oxazolone	decreased	
Fisher-344	1 µg/kg body ueight on gestationdays 11, 18 and on postnatal days 4, 11 and 18 or 5 ug/kg body weight on postnatal days 0, 7 and 14	25 days	lymphoproliferative response of PHA[a]-stimulated spleen cells and of PHA[a]- and ConA[b]-stimulated thymus-cells	decreased	Vos and Moore 1974
			graft versus host assay	decreased	
			skin graft assay	prolonged allograft rejection time	
MICE					
B6C3F$_1$[c]	1, 5 or 15 ug/kg body ueight on gestationday 14 and on postnatal days 1, 7 and 14	NR	bone marrow cellularity	decreased	Luster et al 1980
			stem cell proliferation	decreased	
			lymphoproHferative response of mitogenstimulated spleen cells:		
			PHA[a]	decreased	
			ConA[b]	decreased	
			LPS[d]	no effect	
			Macrophage proliferation	no effect	
			Phagocytizingability	no effect	
			Resistance to Listeria monocyto-genes	decreased	
			Resistance to PYB6 tumor cells	decreased	
Swiss-Webster	Feeding 1, 2.5 or 5 ppb of TCOO in the diet 4 weeks before mating, throughout gestation and 3 weeks postnatally	5-6 weeks	Contact sensitivity to 2,4-dinitro-1-fluorobenzene	decreased	Thomas and Hinsdill 1979
			lymphoproliferative response of PHAa- and ConA[b]-stimulated spleen and thymus cells	no effect	
			Resistance to Salmonella typhi-murium endotoxin	decreased	
			Resistance to Listeria monocyto-genes	no effect	
C57Bl/6Sch	2 or 5 pg/kg body ueight on gestationdays 14 and 17 and on postnatal days 1, 8 and 15	23 days	Skin graft assay	prolonged skin graft rejection time	Vos et al 1974
Swiss	10 ug/kg body weight on postnatal days 1, 4, 8, 11, 15 and 18	22 days	lymphoproHferative response of PHA[a]-, ConA[b]- and PWM[e]-stimulated thymus cells	decreased	Vos et al 1978a

[a] PHA = phytohemagglutinin, [b] ConA = concanavalin A, [c] B6C3F$_1$ = progeny to female C57Bl/6N and male C3H mice, [d] LPS = lipopolysaccharide, [e] PWM poke weed.

Table 8-9. **TCDD-induced** mixed functon oxidases and **UDP-glucuronosyltransferases** in rat **liver microsomes.**

Enzyme	Strain of rat	Total dose of TCDD (pg/kg)	Induction (%)	Reference
Anilinehydroxylase	SD	5		Beatty et al 1978
	CD	25		Hook et al 1975a
	CD	5,25		Lucier et al 1973
Arylhydrocarbon-hydpoxylase	Wistar	20		Aitio and Parkki 1978
	SD	5		Beatty et **al 1978**
	SD	10		Haaparanta et al 1983
	CD	25		Hook et **al** 1975a
	CD	2		Kitchin and Woods 1979
	CD	5,25		Lucier et al 1973
	SD	17		**Manis** and Apap 1979
	Wistar	5		**Nagayama** et al 1983
	SD	10		Thunberg et al 1984
	SD	10		Poland and Glover 1973a, 1974
Biphenylhydroxylase	CD	2		Kitchin and Woods 1979
	CD	25		Hook et **al** 1975a,b
O-deethylase:				
ethoxycoumarin	Wistap	20		Aitio and Parkki 1978
ethoxyresorufin	CD	2		Kitchin and Woods 1979
	SD	10		Haaparanta et al 1983
UDP-glucuronosyl-tpansfepase:				
p-nitrophenol	Wistar	20		Aitio et al 1979
	CD	25		Hook et **al** 1975a
	CD	5,25		Lucier et al 1973, 1975
	SD	10		Thunberg et **al** 1980, 1984
	Wistap	10		Thunberg and **Håkansson** 1983
o-aminophenol	Wistar	20		Aitio et al 1979
4-metylumbelliferon	Wistap	20		Aitio and Parkki 1978

Table 8-10. The potency of various chlorinated cyclic hydrocarbones to reduce hepatic vitamin A content in the rat.

Compound	Dose and route of administration	Duration of the study	X Reduction of hepatic vitamin A	Reference
Arochlor 1242	100 mg/kg in the diet[a]	2 months	49	Cecil et al 1973
p,p'-DDT	100 rig/kg in the diet[a]	2 months	38	Cecil et al 1973
Methoxychlor	10 mg/kg in the diet[b]	16 weeks	7	Davison and Cox 1976
	100		12	
	1 000		37	
	10 000		68	
PCB	100 mg/kg in the diet[c]	8 weeks	82	Innami et al 1976
TCDD	10 µg/kg body weight, single oral dose	7 days	29	Thunberg et al 1979
		14 days	39	
		28 days	59	
		56 days	67	
TCDD	0.1 µg/kg body weight, single oral dose	28 days	2	Thunberg et al 1979
	1.0		27	
	10.0		65	
TCDD	15 µg/kg body weight, single oral dose	44 days	59[d] 88[e]	Håkansson and Ahlborg 1985b
	30		78[d] 98[e]	
	60.		81[d] 97[e]	
	120		90[d] 99[e]	
Toxaphene	20 mg/kg body weight, orally twice weekly	A weeks	0	Thunberg et al 1983

a) 30 400 IE vitamin A/kg diet ad libitum
b) 33 000 IE vitamin A/kg diet ad libitum
c) 3 000 IE vitamin A/kg diet ad libitum
d) 21 000 IE vitamin A/kg diet ad libitum
e) 8 000 IE vitamin A/kg diet ad libitum

Table 8-11. Studies on the potential teratogenic effect of 2,3,7,8-TCDD on rats.

Species/Strain	Vehicle	Daily Dose (route adm)	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Rat/CD	DMSO	0, 0.5, 2.0 ug/kg (subcutaneous)	6-15, 9 and 10, or 13 and 14	20[a]	none reported	kidney malformations at both dose levels	Courtney and Moore 1971
Rat/Sprague-Dawley	corn oil/ acetone	0, 0.03, 0.125, 0.5, 2.0 and 8.0 µg/kg (oral)	6-15	20[a]	vaginal hemorrhage at 2.0 and 8.0 ug/kg	intestinal hemorrhage at 0.125 and 0.5 ug/kg, fetal death at higher doses, subcutaneous edema	Sparschu et al 1971b
Rat/Wistar	corn oil/ anisole	0.0, 0.125, 0.25, 1, 2, 4, 8, 16 µg/kg (oral)	6-15	22	maternal toxicity observed at or above 1 ug/kg	increased fetal death observed at or above 1 ug/kg, subcutaneous edema and hemorrhages in the 0.25-2 ug/kg groups	Khera and Ruddick 1973
Rat/Sprague-Dawley	corn oil/ acetone (9:1)	0.125, 0.5, 2.0 ug/kg (p.o.)	1-3	21	decrease in bw gain in the high dose group	decreased fetal weight in the 0.5 and 2 ug/kg group	Giovani et al 1982a
Rat/Sprague-Dawley	diet	0.001, 0.01 and 0.1 µg/kg[c]	throughout gestation	post-parturition	low fertility at 0.01 and C.1 ug/kg dilated renal pelvis	low survival at 0.01 and 0.1 ug/kg, slight dilated renal pelvis at 0.001 ug/kg in the F_1 but not succeeding generations[d].	Murray et al 1979
Rat/Sprague-Dawley	corn oil/ aceton	0, 0.125, 0.5, 2 ug/kg (p.o.)	Daily 2 weeks before mating	21	0.5 and 2.0 µg decrease in bw	cystic kidney and dilat renal pelvis	Giovani et al 1983

[a] First day of gestation designated day zero.

[b] First day of gestation designated day one.

[c] The high dose level (0.1 µg/kg/day) was discontinued due to very low fertility in adults.

[d] Nisbet and Paxton (1982) re-evaluated the stuy by Murray et al (1979) using different statistical methods and considered the effects in the 0.001 µg/kg group to be statistically significant.

Table 8-12. Studies on the potential teratogenic effect of 2,3,7,8-TCDD on mice.

Species/Strain	Vehicle	Daily Dose (route administration)	Treatment	Observation	Maternal Response	Fetal Response	Reference
Mouse/C57B1/6 Mouse/AKR	DMSO or honey:water (1:1)	21.5, 46.4, 113.0 ng/kg (?)	6-14 or 9-17	19[a]	increased liver/bu ratio	fetocidal, cleft palate, cystic kidney	Courtney et al 1970b
Mouse/CD-1 Mouse/DBA/2J Mouse/C57B1/6J	DMSO	0.5, 1, 3 ug/kg (subcutaneous)	6-15	17[a] or 18	increased liver-ueight/bu ratio	cleft palate, kidney anomalies	Courtney and Moore 1971
House/C57B1/6	acetone: corn oil (1:9)	1, 3 ug/kg (oral)	10-13 or 10	18[a]	none reported	cleft palate, kidney anomalies	Moore et al 1973
Mouse/CD-1	DMSO or corn oil	25, 50, 100 200, 400 ug/kg (oral + sub-cutaneous)	7-16	18[b]	increased liver/bu ratio	cleft palate, hydro-nephrotic kidneys, hydrocephalus, open eyes, edema, petechiae	Courtney 1976
Mouse/CF-1	corn oil/ acetone (98:2)	0.001, 0.01 0.1, 1.0, 3.0 ug/kg (oral)	6-15	18[a]	none reported	cleft palate, dilated renal pelvis	Smith et al 1976
Mouse/NMRI	rape-seed oil	0.3, 3.0, 4.5 9.0 ug/kg (oral)	6-15	18	no effect observed	fetocidal at the high dose, cleft palate at doses at or above 5 ug/kg	Neubert and Dillman 1972

TABLE 8-13. Carcinogenicity Bioassays of PCDD Administration by the Oral Route

Exposure Route/ Compound	Species/Strain	Sex	Dose of Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	Reference
Oral/ 2,3,7,8-TCDD	rat/ Sprague-Dawley	M	0.0 ppb	78 weeks	95 weeks	in diet	all tumors	0/10	Van Miller et al 1977
			0.001 ppb	78 weeks	95 weeks	in diet	all tumors	0/10	
			0.005 ppb	78 weeks	95 weeks	in diet	all tumors	5/10	
			0.05 ppb	78 weeks	95 weeks	in diet	all tumors	3/10	
			0.5 ppb	78 weeks	95 weeks	in diet	all tumors	4/10	
			1.0 ppb	78 weeks	95 weeks	in diet	all tumors	4/10	
			5.0 ppb	78 weeks	95 weeks	in diet	all tumors	7/10	
Oral/ 2,3,7,8-TCDD	rat/ Sprague-Dawley	M	0.0 µg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate, squamous cell carcinoma of the tongue, adenoma of the adrenal cortex	0/85 0/85 0/85	Kociba et al 1978
			0.001 µg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate, squamous cell carcinoma of the tongue, adenoma of the adrenal cortex	0/50 1/50 0/50	

Oral/ 2,3,7,8-TCDD	rat/ Sprague-Dawley	M	0.01 yg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate, squamous cell carcinoma of the tongue, adenoma of the adrenal cortex	0/50 1/50 2/50	Kociba et al 1978	
			0.1 yg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate, squamous cell carcinoma of the tongue, adenoma of the adrenal cortex	4/50 3/50 5/50		
Oral/ 2,3,7,8-TCDD	rat/ Sprague-Dawley	F	0.0 yg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma, squamous cell carcinoma of the tongue, squamous cell carcinoma of the lung	0/86 0/86 0/86	Kociba et al 1978	
			0.001 yg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma, squamous cell carcinoma of the tongue, squamous cell carcinoma of the lung	0/50 0/50 0/50		

			0.01 yg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma,	2/50		
							squamous cell carcinoma of	1/50		
							the tongue,	0/50		
							squamous cell carcinoma of the lung			
Oral/ 2,3,7,8-TCDD	rat/ Sprague-Dawley	F	0.1 yg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma,	11/49	Kociba et al 1978	
							squamous cell carcinoma of	4/49		
							the tongue,	7/49		
							squamous cell carcinoma of the lung			
Gavage/ 2,3,7-8-TCDD	mice/Swiss/ H/Riop	M	0.0 yg/kg/week	365 days	588 days	sunflower oil	liver tumors	7/38	Toth et al 1979	
			0.007 yg/kg/week	365 days	649 days	sunflower oil	liver tumors	13/44		
			0.7 yg/kg/week	365 days	633 days	sunflower oil	liver tumors	21/44		
			7.0 yg/kg/week	365 days	424 days	sunflower oil	liver tumors	13/43		
Gavage/ 2,3,7,8-TCDD	rats/ Osborne-Mendel	M	0.0 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	1/69	NIH 1982a	
			0.1 yg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	5/48		
			0.05 yg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	8/50		

			0.5 yg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas or carcinoma of the thyroid	11/50	
Gavage/ 2,3,7,8-TCDD	rats/ Osborne-Mendel	F	0.0 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	5/75	NIH 1980
			0.1 lag/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	1/49	
			0.05 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	neoplastic nodule or hepatocellular carcinoma of the liver	3/50	
			0.5 µg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	rteoplastic nodule or hepatocellular carcinoma of the liver	14/49	
Gavage/ 2,3,7,8-TCDD	mice/B6C3F1	M	0.Q yg/kg week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	8/73	NIH 1982a
			0.1 yg/kg week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	9/49	
			0.05 yg/kg week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	8/49	
			0.5 yg/kg week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	17/50	

Gavage/ 2,3,7,8-TCDD	mice/B6C3F1	F	0.0 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	1/73 0/69	NIH 1982a
			0.04 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	2/50 3/50	
			0.2 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	2/48 1/47	
			2.0 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	6/47 5/46	
Gavage/HxCDD 1,2,3,6,7,8/ 1,2,3,7,8,9 (1:2)	rats/ Osborne-Mendel	N	0.0 yg/kg/week (vehicle control)	104 weeks	105 weeks	corn oil-acetone	liver neoplastic nodules or hepatocellular carcinoma	0/74	NIH 1980
			1.25 yg/kg/week	104 weeks	106 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	0/49	
			2.5 yg/kg/week	104 weeks	107 weeks	corn oil-acetone	liver neoplastic nodules or hepatocellular carcinoma	1/50	
			5.0 yg/kg/week	104 weeks	107 weeks	corn oil-acetone	liver neoplastic nodules or hepatocellular carcinoma	4/48	

Gavage/HxCOD 1,2,3,6,7,8/ 1,2,3,7,8,9 (1:2)	rats/ Osborne/Mendel	F	0.0 yg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	5/75	NIH 1980
			1.25 ug/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	10/50	
			2.5 yg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	12/50	
			5.0 yg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	liver neoplastic nodules or hepatocellular carcinoma	30/50	
Gavage/HxCDD 1,2,3,6,7,8/ 1,2,3,7,8,9 (1:2)	mice/B6C3F1	M	0.0 yg/kg/week	104 weeks	105 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	15/73	NIH 1980
			1.25 yg/kg/week	104 weeks	108 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	14/50	
			2.5 yg/kg/week	104 weeks	107 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	14/49	
			5.0 yg/kg/week	104 weeks	108 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	24/48	
Gavage/HxCOD 1,2,3,6,7,8/ 1,2,3,7,8,9 (1:2)	mice/B6C3F1	F	0.0 yg/kg/week	104 weeks	106 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	3/73	NIH 1980

			2.5 yg/kg/week	104 weeks	108 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	4/48	
			5.0 yg/kg/week	104 weeks	108 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	6/47	
			10.0 yg/kg/week	104 weeks	108 weeks	corn oil-acetone	hepatocellular adenomas or carcinomas	10/47	
Oral/ 2,3,7,8-TCDD	mice/ Peramyscus polienotus	M&F	0.0012 yg/kg/day	Field exposure		contaminated soil	liver	0/15	Cockerham et al 1980
			0.0 yg/kg/day	Field exposure		contaminated soil	liver	0/15	

Table 8-14. Structure-activity relationships for some PCDDs.

Chlorination of PCDDs	AHH-induction potencies (nM)[a]	Receptorbinding avidities (nM)[b]	LD_{50} guinea pig (µg/kg body weight)
2,8	inactive[d]		>300 000
2,3,7	1 100	1.9	29 400
2,3,7,8	0.4	0.27	2
1,3,7,8	89	1.7	
1,2,3,8	610		
1,2,3,7,8	5.4	0.42	3.1
1,2,4,7,8	>120 000[e]		1 125
1,2,3,4,7,8	7.6		72.5
1,2,3,6,7,8	31	0.57	70-100
1,2,3,7,8,9	46	1.4	60-100
1,2,3,4,6,7,8	130		>600
1,2,3,4,6,7,9	3 700		
1,2,3,4,6,7,8,9	19 000		

a) Estimated concentration needed to produce 50% maximum enzyme induction in the rat hepatoma cell line H-4-II-E (Bradlaw and Casterline 1979).
b) Estimated concentration needed to displace 50% of ^{3}H-TCDD bound to liver cytosol receptor from C57Bl/6J mice (Poland et al 1976).
c) McConnell et al 1978.
d) Inactive at the highest dose tested
e) Calculated value

Table 8-15. Structure-activity relationships for some PCDFs.

Chlorination of PCDFs	AHH-induction potencies (nM)[a]	Receptorbinding avidities (nM)[b]	LD_{50} guinea pig (µg/kg body weight)[c]
2,8	39 500	257 000	
2,3,8	2 490	1 000	
2,3,7,8	3.9	41	>5 <10
2,3,6,8	1 040	2 200	
2,3,4,7,8	0.25	15	>5 <10
1/2,3,7,8	2.5	75	
1,2,4,7,8	106	1 300	
2,3,4,6,7,8	0.70	47	
1,2,3,4,7,8	0.36	230	
1,2,3,6,7,8	1.5	270	

a) Estimated concentration needed to produce 505! maximum enzyme induction in the rat hepatoma cell line H-4-II-E (Bandiera et al 1984)
b) Estimated concentration needed to displace 50% of ^{3}H-TCDD bound to liver cytosol receptor from Wistar rats (Bandiera et al 1984)
c) Moore et al 1979

Table 9-1

Accidents in Chemical PLants Involving the Manufacture of Chlorinated Phenolic Compounds.

Me	Country	Manufacturer/location	Product	Cause of exposure	Personnel affected	Veers from incident to last observation	References
1910	fcmuny	Leverkusen	TCDD	Explosion + occupational	5	Saw year	Teleky, 1913; Wahle, 1914; tahneier and Jamson, 1983
1949	United States	Monsanto/Nitro, West Virginia	TCP	Explosion + Occupational	228	30	Ashe and Suskind, 19*9, 1950; Huff ft al, 1980; Moses et al, 1984, Suskind, 1978; Suskind et al, 1951, 1984; Zack and Gaffey, 1983; Zack et al, 198*
1949	Federal Republic of Germany	Nordrhein-Westfalen	TCP (PCP)	Occupational	17	1	Baader et al, 1951
1952	Federal Republic of Germany	Nordrhein-Westfalen	TCP	Occupational	60	-	Bauer et al, 1941
1952-1953	Federal Republic of Germany	Boehringer	TCP	Occupational	37	-	Hay, 1977
1953	Federal Republic of Germany	BASF/Ludwigshafen	TCP	Explosion + Occupational	75	29	Goldmann, 1972, 1973; rtoffwn, 1957; Huff « al, 1980; Thiess et al, 1976, 1977, 1982
1953-1971	France	Rhone Poulenc/Grenoble	TCP	Occupational « explosion	17	2	Dugois et al, 1956, 1957, 1958
1954	Federal Republic of Germany	Boehringer/Ingelheim Hamburg	TCP, 2,4,5-T	Occupational	31	2*	Kimmig et al, 1957a,b; Bluer et al, 1941; Kleu et al, 1971; von Krause et al, 1978
1954	Federal Republic of Germany	?	TCP 2,4,5-T	Occupational	2*	*	Risse-Sundermann, 1959
1956	United States	Diamond Alkali/ Newark, New Jersey	2,4,5-T 2,4,5-T	Occupational	(8	6	Bleiberg et al, 1964; Poland et al, 1971
1956	United States	Hooker/Niagara Falls, New York	TCP	Occupational	Many	-	Hay, 1977
1959	Italy	Industrie Chimiche Melegnanesi Saronio/ Milan	TCP	Explosion + Occupational	5	2	Hofmun et al, 1962
1959	United States	Thompson-Hayward/ Kansas City, Kansas	TCP	Occupational	-	??	Hey, 1977
1960	United States	Diamond Shamrock/ Newark NJ	TCP	Occupational	many	-	Hay, 1977
1963	Netherlands	Philips-Duphar/ Amsterdam	TCP 2,4,5-T	Explosion	106	11	Berlin et al, 1976; Calderup, 1974a,b; Huff et al, 1980
194*	USSR		2,4,5-T	Occupational	128	-	Telegina et al, 1970; IARC, 1977
1964	United States	Dm Chemical/ ftidlard, Michigan	TCP	Occupational	61	6	Cook et al, 1980; Firestone, 1980; Ott et al, 1980; Vahrenholt, 1977 Rowe 1980
1964-1969	Czechoslovakia	Spolana	TCP	Occupational	80	6	Jirasek et al, 1973, 1974, 1976; Pazderova et al, 1974, 1980, 1981
1968	United Kingdom	Coalite and Chemicals Products/Bolsover, Derbyshire	TCP	Explosion	90	14	Hay, 1973, 1982; Huff et al, 1980
1970	Japan		2,4,5-T	Occupational	25	3	Miura, 1974
1972	USSR		TCP	Occupational	1	1	Zelikov, 197*
1972-1973	Austria	Österreichische Stickstoffwerke/Linz	2,4,5-T	Occupational	SO	-	Forth, 1977; Hey, 1977
197*	Federal Republic of Germany	Bayer/Uerdingen	2,4,5-T	Occupational	5	-	Forth, 1977; Hay, 1977
19%	Italy	ICMESA/Meda/Seveso	TCP	Explosion	193	8	Basso et al, 1982; Filippini et al, 1981; Ideo et al, 1983; Kameda, 1977; Reggiani, 1977a,b,c, 1978a,b, 1983; Vahrenholt, 1977

Table 9-2. Human Illness Associated with Arena Soil Exposure

Case	Arena	Age, sex		Illness
1	A	6	F	haemorrhagic cystitis, epistaxis/ headache, diarrhoea, lethargy
2	A	10	F	epistaxis, headache, diarrhoea, skin lesions, "arthralgia"
3	A	adult	M	headache, "arthralgia"
4	A	adult	F	headache, diarrhoea, skin lesions
5	D	3	M	skin lesions
6	D	3	M	skin lesions

Table 9-3

SIGNS AND SYMPTOMS REPORTED IN ASSOCIATION WITH EXPOSURE TO TCDD OR MIXTURES CONTAINING TCDO

A. Skin Manifestations

1. Chloracne
2. Hyperkeratosis
3. Hyperpigmentation
4. Hirsutism
5. Etastosis

B. Systemic Effects

1. Mild fibrosis of liver
2. Raised transaminase values in blood
3. Hypercholesterolemia
A. Hypertriglyceridemia
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

C. Neurological Effects

1. Sexual dysfunction
2. Headache
3. Neuropathy
A. Sight disturbance
5. Loss of hearing, taste and smell

D. Psychiatric Effects

1. Sleep disturbance
2. Depression
3. Loss of energy and drive
A. Uncharacteristic bouts of anger

Comments to table 9-3

A. Skin manifestations

Chloracne is a sign of exposure to several chlorinated cyclic organic compounds, the most potent is TCDD. Heavy exposure to these compounds is believed always to produce chloracne. Chloracne thus serves as a sensitive marker of such exposure.

While the absence of chloracne does not absolutely negate exposure to a dose of TCDD, its absence usually indicates that there has been no exposure to a toxic dose of the substance. By "toxic" is meant both systemic and local effects. Where there has been exposure to TCDD and chloracne has resulted, it is the only clinical sign which persists for a long period. It may persist for the course of the exposed person's life. In a large group exposed to mixtures containing TCDD, the absence of chloracne:

a) makes it improbable that there was exposure to a toxic dose;
b) renders it unlikely that systemic disorders will result.

Hyperkeratosis is a fairly common fenomenon whereas hyperpigmentation and hirsutism is rare. It should be noted that hyperkeratosis is prominent in the Seveso children who have no affected sebaceous glands. The latter only develop at puberty.
Elastosis *has* been noted as a longterm effect.

B. Systemic effects

Affection of the liver has been diagnosed even by histological examination and accounts for temporarly raised transaminases in blood, hypercholesteremia and hypertriglyceridemia. Bauer et al (1961) and Risse-Sundermann (1959) do not however exclude a virus hepatitis. Loss of appetite, weight loss and digestive disorders are common complaints both by the rare occasions to "pure" TCDD exposure and in the exposure to technical mixtures.

Muscular aches and pain and extremity weakness has been reported particularily after exposure to technical mixtures.
Swollen lymph gland has been reported both after exposure to "pure" TCDD and to mixtures. Cardiovascular, urinary tract, respiratory and pancreatic disorders reported are of doubtful significance with regard to their relationship to TCDD exposure.

Porphyria cutanea tarda has been reported in two cases of occupational exposure where other chlorinated organic compounds were manufactured in addition to trichlorophenol, namely the incident at the factory of Diamond Alkali, Newark, New Jersey in 1956, and the incident at Spolana, Czechoslovakia between 1964 and 1969. The porphyria cutanea tarda observed in those cases very likely was not induced by exposure to TCDD, but rather by exposure to other chlorinated organic compounds manufactured.

C. Neurological effects

Sexual dysfunctions (lack of libido and impotence) have been reported after acute exposure to both "pure" TCDD and technical mixtures. The frequency of its occurrence is most likely underestimated. Headache is a frequent symtom in exposure to technical mixtures.

Sensory neuropathy has been noted in many instances. Usually workers will complain of pains in their joints, particularly early on, after they have very acute severe chloracne; however, there are usually no abnormal physical findings in the joints but the complaints may be lasting. In the early studies of the affected workers no attempts were made to objectively measure the effects on the sensory nervous system. Tests have now been developed which evaluate sensory nerves and which can be used in field studies. The nerve conduction tests, which primarily have been used thus far, are actually not very useful to measure neuropathy. Differences in nerve conduction were shown among residents from Seveso, Italy who had chloracne and residents who did not (Fillipini et al 1981). A recent workshop of the Worl Health Organization (WHO) has addressed the subject of standardizing neurological examinations. This workshop will be published with recommendations on how to evaluate the

nervous system. Testing nerve conduction velocity would not be very useful in determining whether a sensory neuropathy was present. El Batawy, MA. Report on neurological effects. Cheif Medical Officer, Occupational Health, WHO.

Sight disturbance may be related to alkaline exposure or: to conjunctivitis related to the affection of the glands of Meibom. Loss of hearing, taste and smell have been reported in a few cases. Their relationship to TCDO is doubtful.

D. Psychiatric effects

The symtoms have been listed in what is beleived to be there frequency and degree of severity.

Sensitivity to TCDD Han and animals

Although few quantitative measurements are available a study of the episodes of human exposure presented in chapter 9 and in table 9-1 leads one to beleive that man is not very sensitive to TCDO as compared to animals. Chloracne, which does not occur in animals, is an exception. In no case has death been reported in immediate relation to exposure although people have been killed by impact of explosion or suffocation. The contamination of plant personnel has been considerable and the technical mixture has been applied to volunteers and unexposed skin of exposed workers.

In two instances workers without much or insufficient protection have climbed into and cleaned the reactor that contained the remains of the reaction (Monsanto 1949 and Industrie Chimiche Melegnanesi 1959). Although suffering from chloracne these people are still alive. Pure TCDD has also been applied to the skin of man in amounts up to 7.5 mg without any reported untoward systemic effects. Sandermann had 20 g of TCOD in an open container on his desk for 2 weeks and his coworker opening a dessicator without equalizing the pressure must have been exposed to more than mg quantities.

By contrast some animals are particularily sensitive to TCDD (table 00). In several instances rodents exposed to the atmosphere of working conditions or general surrounding have died in contrast to man (BASF, Spolana, Coalite, Philips-Duphar and ICMESA).

Table 10-1. Distribution of radioactivity to the major tissues depots at various time-points after an i.v. dose of ^{14}C-2,3,7,8-TCDF to rat[a], mouse[b], guinea pig[c] and monkey[d] along with the respective calculated values for uhole body half-life and LD_{50}.

	Fisher 344 rats (30.6 µg/kg)			C57Bl/6J mice (30.6 µg/kg)			DBA/2J mice (30.6 µg/kg)			Hartley guinea pigs (6 µg/kg)			Rhesus monkey (30.6 µg/kg)
	3 hr	3 d	10 d	3 hr	3 d	10 d	3 hr	3 d	10 d	3 hr	3 d	9 d	21 d
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	23.6+3.8	29.3+0.6	54.2±14.5	1.02+0.80
Fat	10.0+1.0	11.1+2.3	1.8	6.0+1.6	2.9+2.1	NO	9.6*3.9	22.3+2.9	7.2*0.9	31.4±0.7	56.9+7.6	21.8*11.6	3.66+2.83
Skin	6.640.3	1.2+0.3	0.5	3.6±0.7	3.0+1.1	ND	5.5*4.3	3.3+0.9	ND	22.5+0.1	17.140.6	15.2*3.1	2.44*1.60
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		15.6*4.5	8.8±3.0	1.55+0.14
Feces		63.1+0.6	>85		43.1	81 .9±13.0		27.7	55.8±4.8		4.7*1.3	6.6	42.9
Urine		2.0+0.4	<6		7.7	12.6+0.1		9.2	19.9+4.6		2.3*0.4	6.6	7.9
t1/2 (days)		<2[b]			2[b]			4[b]			>20[c]		8[a]
LD_{50} (µg/kg)		>6000[e]			>6000[e,f]						>5[c] <10[e,f]		1000[f]

[a] 30.6 µg/kg (Bimbaum et al 1980)

[b] 30.6 µg/kg (Decad et al 1981b)

[c] 6 µg/kg (Decad et al 1981a)

[d] 30.6 µg/kg (Bimbaum et al 1981)

[e] Moore et al 1976

[f] Moore et al 1979

ND - not detectable

Table 10-2. Yusho-Japan. Intake of contaminated rice oil.

Hayabuchi et al (1979)

Total rice oil consumed: 195-3375 ml X=688 ml

Total estimated intake:	PCBs	633 mg
	PCDFs	3.4 mg
	PCQs	596 mg
Estimated daily intake:	PCBs	157 yg/kg/day
	PCDFs	0.9 yg/kg/day
	PCQs	148 yg/kg/day

Masuda and Kuroki (1982)

Rice oil contained	PCBs	1000 ppm
	PCDFs	5 ppm
	PCQs	900 ppm

Yoshimura et al (1971);

estimated rice oil intake	<500 ml
	500-999 ml
	>1000 ml

Nagayama et al (1975); rice oil contained 5 ppm PCDFs.

Masuda et al (1982): rice oil contained 2 ppm PCDFs.

Table 10-3. Yu-Cheng - Taiwan. Intake of contaminated rice oil,

Chen et al (1981)
(Bull Environ Contam Toxicol 26, 489-495)

rice oil contained:	PCBs	53-99 ppm
	PCDFs	0.18-0.40 ppm
	PCQs	25-53 ppm

Lan et al (1981)

Estimated oil intake (kg/person and month) = 1.02 - 1.55

Estimated total oil intake (kg/person) = 9-14

Estimated intake of:	PCBs	973 mg
	PCDFs	3.8 mg
	PCQs	586 mg

Miyata et al (1985)

Rice oil contained average:	PCBs	62 ppm
	PCDFs	0.14 ppm
	PCQs	20 ppm

Hsu et al (1984)

Estimated PCB intake 0.7 - 1.84 g/person.

Chen et al (1985)

Rice oil contained:	PCBs	53-99 ppm
	PCDFs	0.18-0.40 ppm
	PCQs	25-53 ppm

Table 10-4 Clinical symptomatology of Yusho 1969-1972.

1. Skin (82-87%).
   Acneform eruptions, districtive hair follicles, red plaques on limbs, dark brown pigmentation of nail, skin and mucous membranes, itching, sweating of palms.

2. Ocular manifestation (83-88%).
   Increased eye discharge, swelling of the upper eyelide, hyperemia of conjunctives, transient visual disturbance.

3. Jaundice (10%).
   No abnormalities of liver functions in the majority of cases.

4. Numbness of the limbs, feeling of weakness, spasm of the muscles (32-39%).
   Reduced sensory and motor nerve conduction velocity in few cases (9%).

5. Hearing difficulties (18%).

6. Headaches, vomiting, diarrhoea (17-39%).

7. Chronic bronchitis (40%).
   Lower serum IgA and IgM, PCB in the sputum.

8. Irregular menstrual cycles (60%).

9. Darkbrown skin pigmentation of newborn, which gradually faded away, retarded growth and abnormal teeth number and shape.

Table 10-5. Changes in the clinical symptomatology of Yusho in the years 1968-1978.

1. Skin lesions.
   All skin symptoms have diminished gradually, subcutaneous cyst formation still occurring in some of the most severe cases.

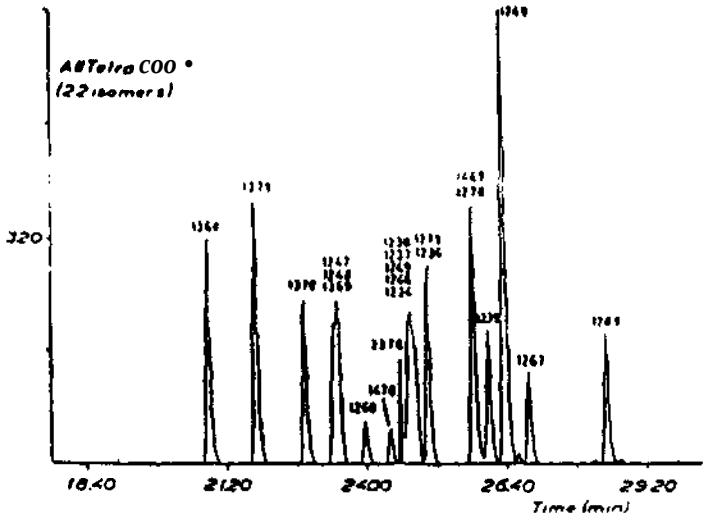
2. Ocular manifestations.
   Eye discharge, oedema of the eyelide, pigmentation of eyelide and conjunctiva, cyst formation of tarsal gland, still present in some of the cases.

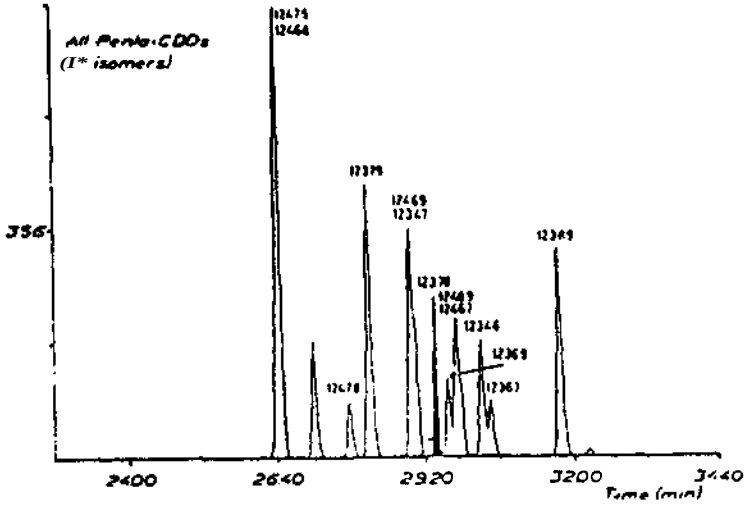
3. Stomatological alterations.
   Pigmentation of oral mucosa is gradually decreasing, anomalies in number of teeth and shape of the root are still present.

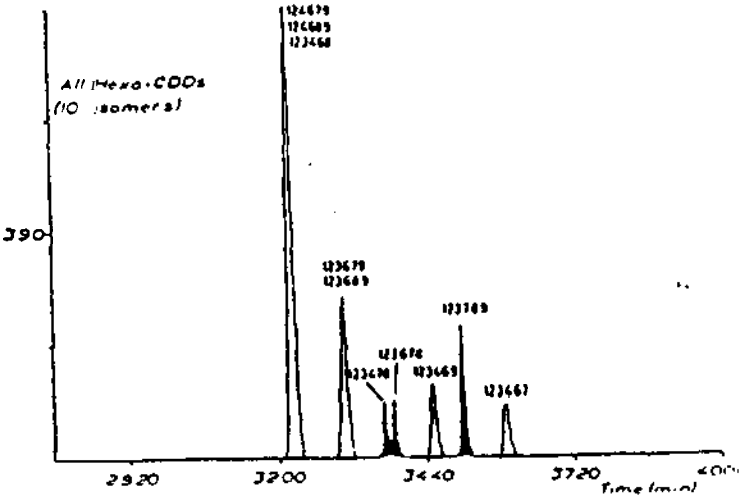
4. Chronic bronchitis correlated in severity with concentration of PCBs in sputum and blood.

5. Serum triglycerides.
   The hyperglycidemia observed in 1968-1970 has lowered to normal values since 1973 in females and since 1975 in males.

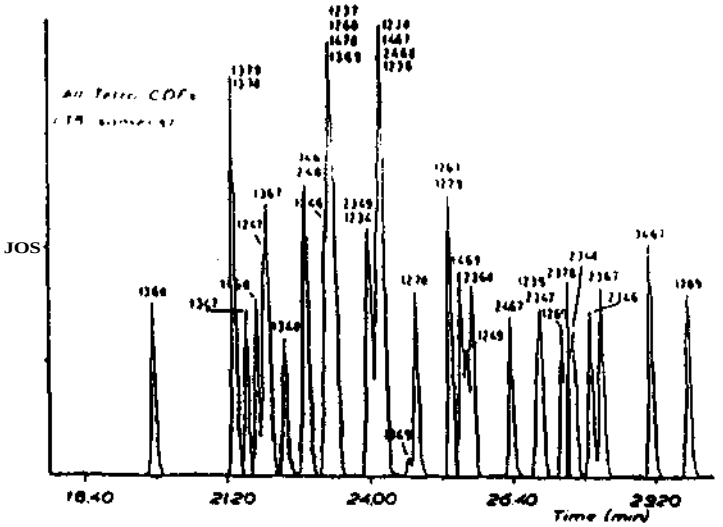
6. Mortality:
   of 737 cases of Fukuota region.
   51 have died (6.92%) during the ten years. There have been 11 cancer death (3 stomach cancer, 2 lung cancer, 1 breast cancer, 1 liver cancer, 2 malignant lymphoma).

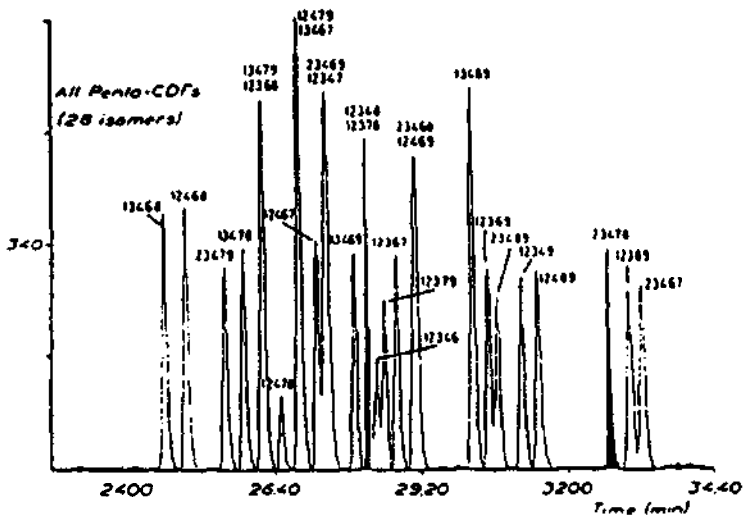






Fig, 3-1. Separation of the 22 tetra-, 14 penta- and 10 hexa-CDDs on a 60 m SP 2330 column.





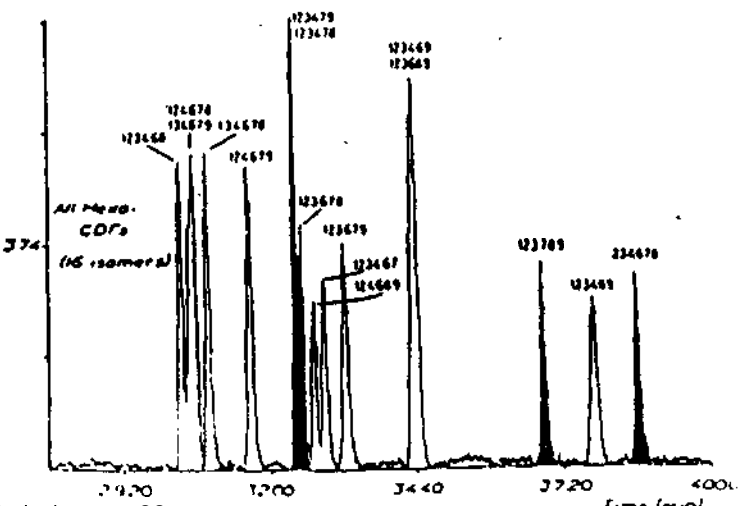


Fig. 3-2. Separation of the 38 tetra-, 28 penta- and 16 hexa-CDFs on a 60 m SP 2330.

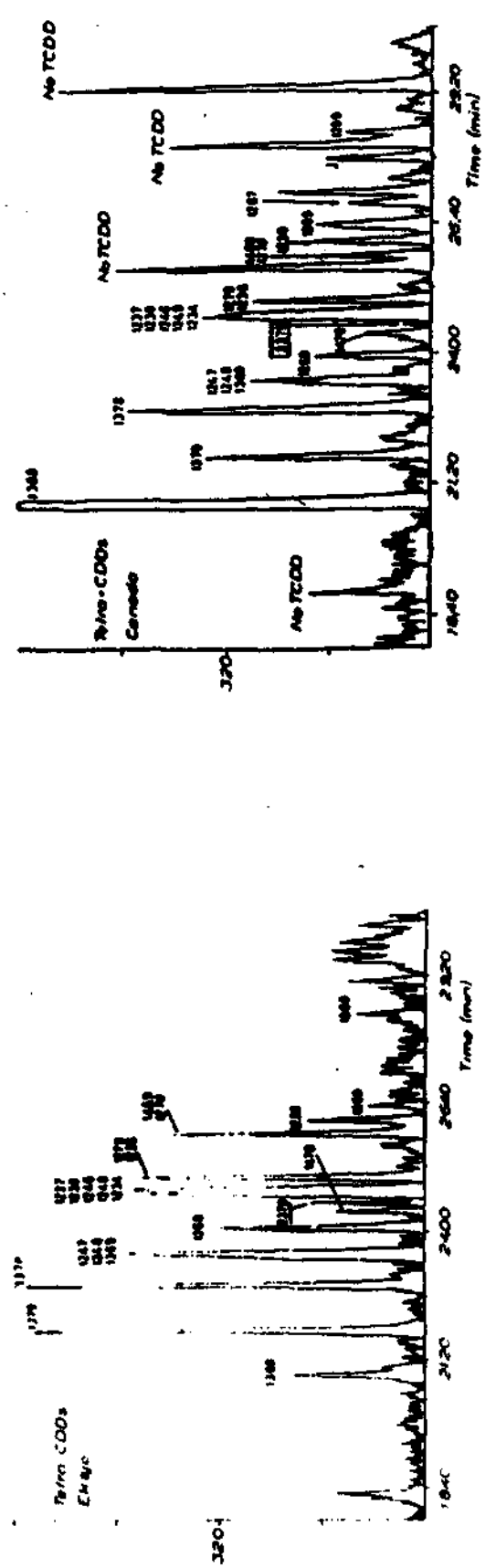
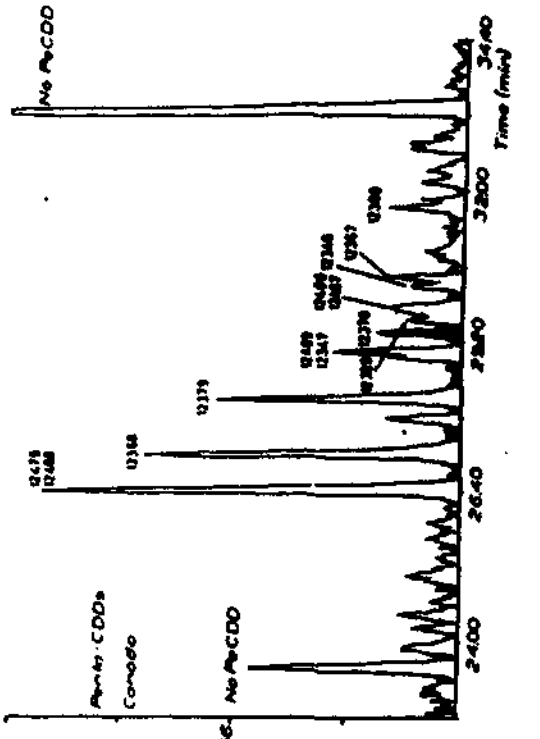


Figure 4-4



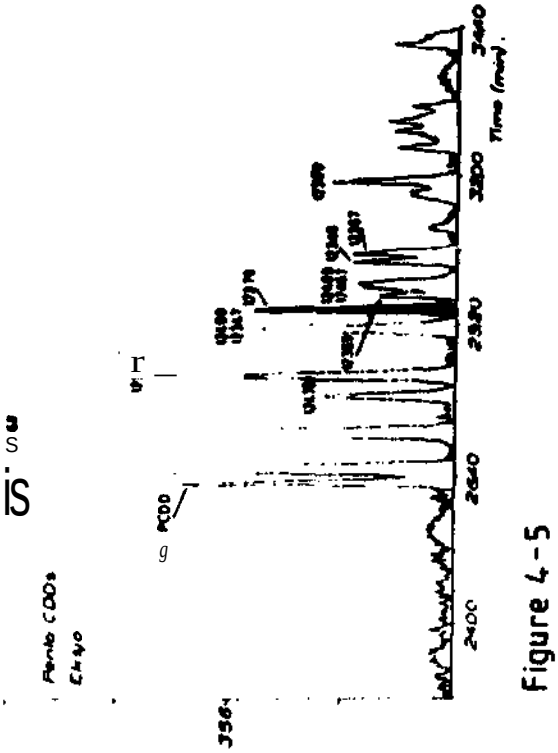


Figure 4-5

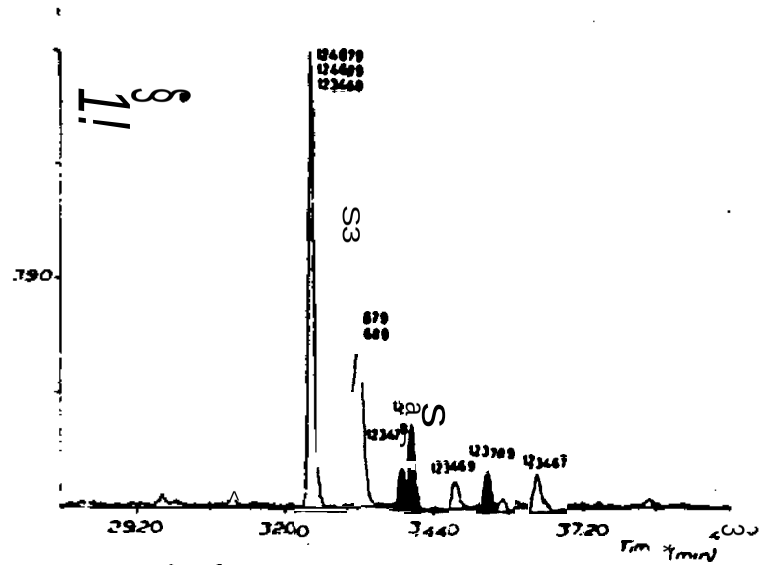
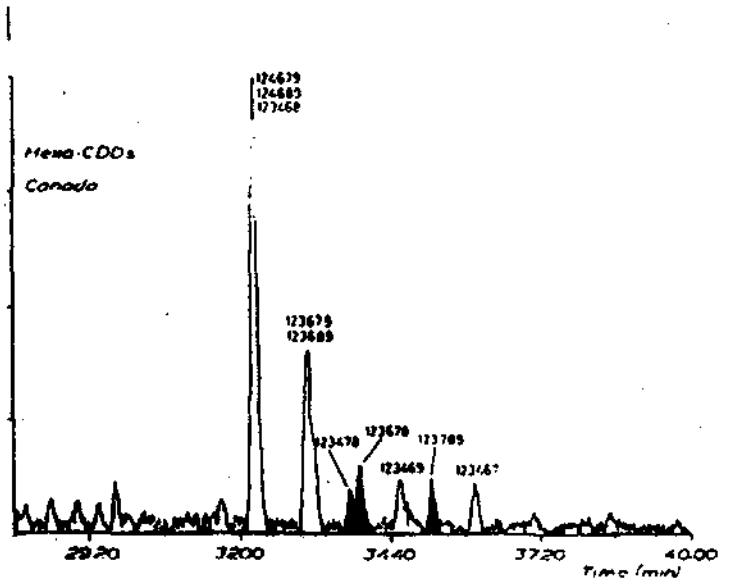


Figure 4-6



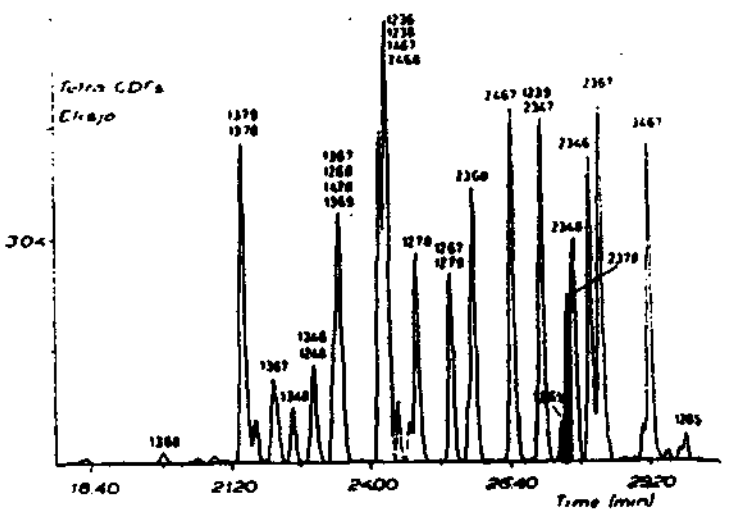
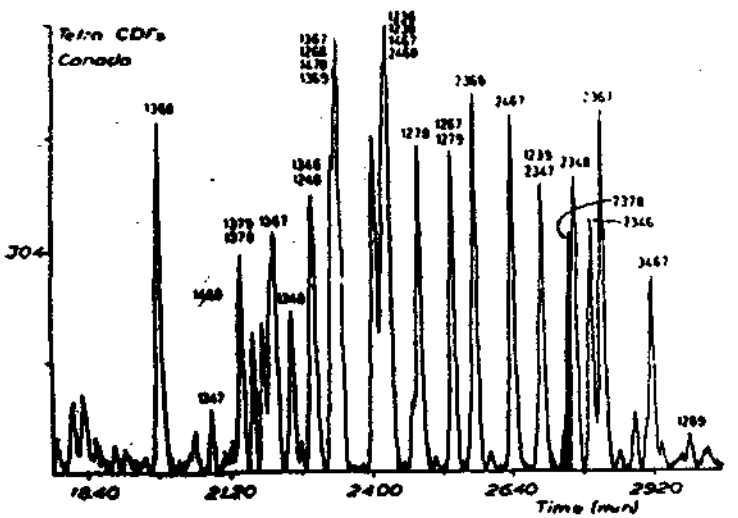
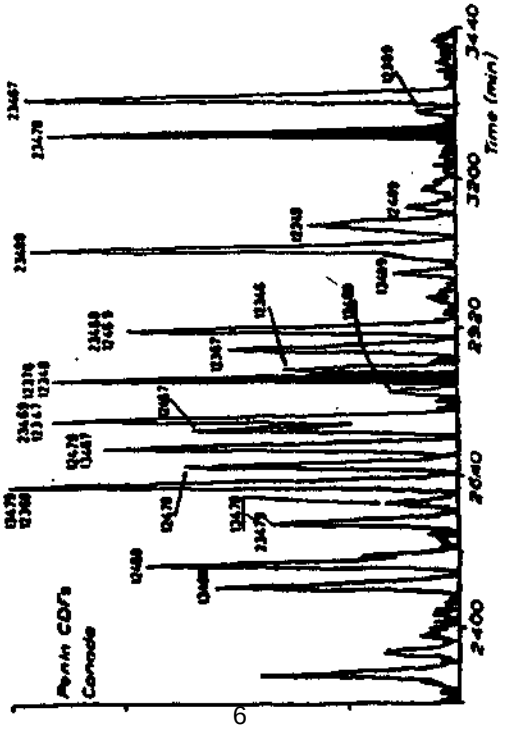


Figure 4-7





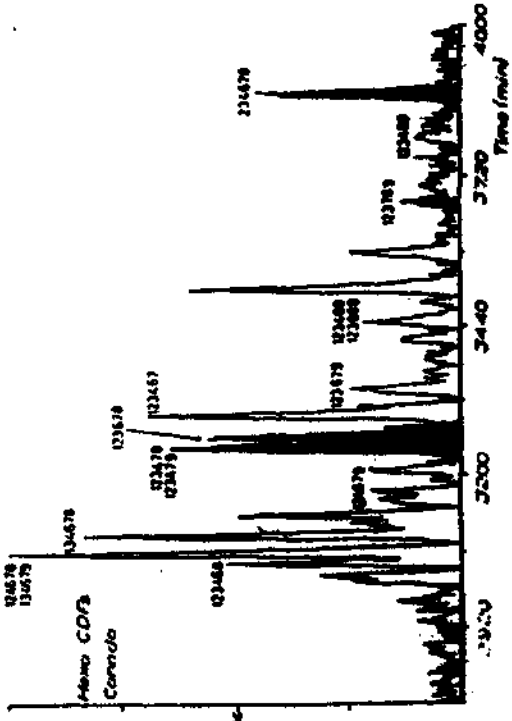
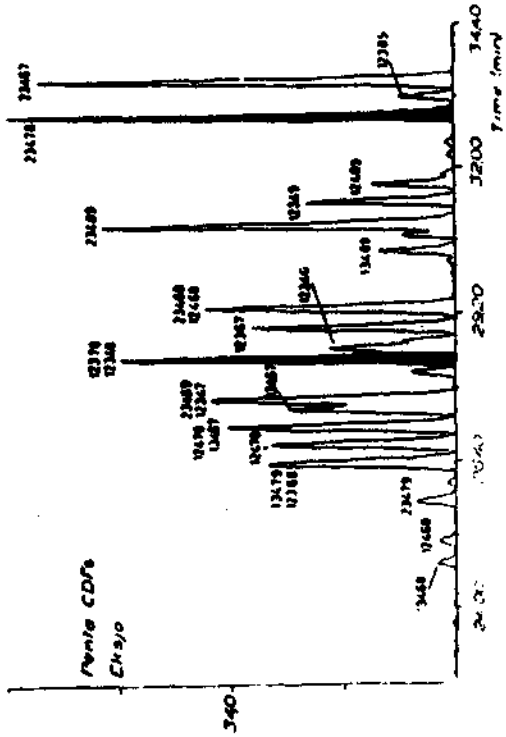


Figure 4-8



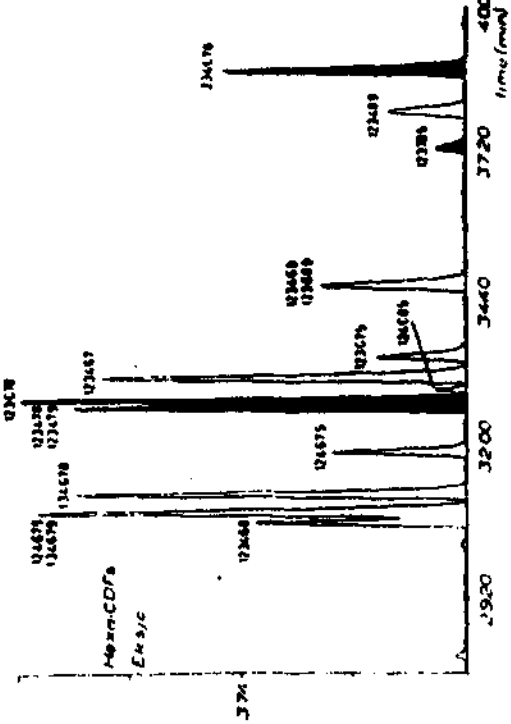
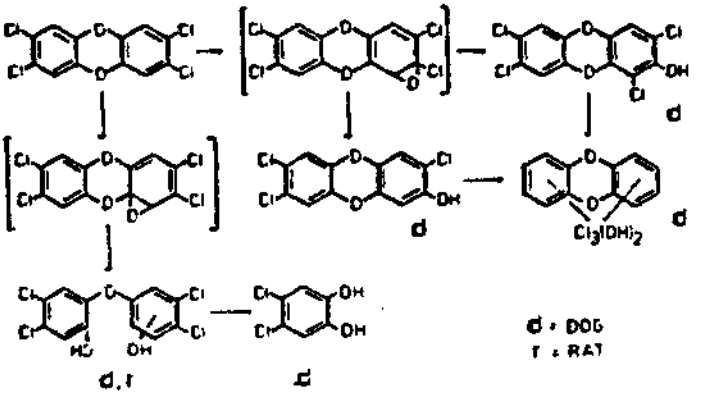


Figure 4-9

Figure 7-1. Proposed metabolic breakdown scheme of TCDD (Poiger and Buser 1982)



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