Danish Ministry of the Environment
Environmental Protection Agency

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs)

Evaluation of health hazards and estimation of a quality criterion in soil

Environmental Project No. 1521, 2013

Title:

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). Evaluation of health hazards and estimation of a quality criterion in soil

Author:

John Christian Larsen
Pia Nørhede
Division of Toxicology and Risk Assessment
National Food Institute, Technical University of Denmark

Published by:

The Danish Environmental Protection Agency
Strandgade 29
1401 Copenhagen K
Denmark
www.mst.dk/english

Year:

Authored in 2004
Published in 2013

ISBN no.

978-87-93026-76-6

Contents

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) and a proposal of health based quality criteria for soil. This resulted in 2004 in the present report, which was prepared by John Christian Larsen and Pia Nørhede, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Nature Agency,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
Danish Regions (former Amternes Videncenter for Jordforurening),
The Danish Environmental Protection Agency.

The Danish Environmental Protection Agency
Copenhagen, December 2013.

1 General description

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are compounds in which 1-10 chlorine atoms are attached to one of three different structures containing two benzene rings. There are 75 possible polychlorinated dibenzo-*p*-dioxins. They are referred to as congeners of one another. There are 135 possible polychlorinated dibenzofuran congeners and 209 possible polychlorinated biphenyl congeners (ATSDR 1997, IARC 1997).

In the next chapters, information about the three groups of chemicals are listed in the following order:
a) Polychlorinated dibenzo-*p*-dioxins
b) Polychlorinated dibenzofurans
c) Polychlorinated biphenyls

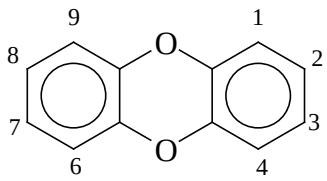
Several risk assessments of exposure to PCDDs, PCDFs and PCBs have been performed recently. This assessment in particular make use of the monographs published by the World Health Organization (WHO 1998), the EC Scientific Committee for Food (SCF 2000, 2001) and the Joint FAO/WHO Expert Committee for Food Additives (JECFA 2002).

1.1 Identity

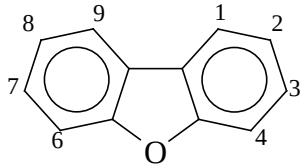
Molecular formulas:
a) $C_{12}H_{8-n}Cl_nO_2$; n = 1-8
b) $C_{12}H_{8-n}Cl_nO$; n = 1-8
c) $C_{12}H_{10-n}Cl_n$; n = 1-10

Structural formulas:

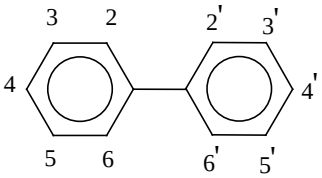
a)



b)



c)



Molecular weights:
a) 219 - 460
b) 203 - 444
c) 189 - 499

Synonyms:
a) PCDDs, dioxins
b) PCDFs, furans
c) PCBs

PCDDs, PCDFs and PCBs exist in environmental and biological samples as complex mixtures of various congeners. A number of the PCDDs and PCDFs, as well as some co-planar PCBs (dioxin-like PCBs) have been shown to exert a number of toxic responses similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-tetraCDD), which is the most toxic dioxin (Van den Berg et al. 1998). The toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, reproductive and developmental toxicity and most, if not all, are mediated *via* the aryl hydrocarbon (Ah) receptor present in most tissues of animals and humans. The toxicity of the individual congeners differs considerably. The dioxin and furan congeners that are of toxicological importance are substituted in each of the 2-, 3-, 7- and 8-positions. Thus, from 210 theoretically possible congeners, only 17 are of toxicological concern. These compounds have a similar toxicological profile to that of the most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD).

The co-planar PCBs are those where the benzene rings can rotate around the bond connecting them. This is mainly possible for congeners with no or only one chlorine atom in positions 2, 2', 6 and 6' (ortho positions) since the steric hindrance will be minimal. These dioxin-like PCBs only constitute a minor fraction of the total amount of a PCB mixture (total-PCB) (ATSDR 1997). Of the 209 theoretical PCBs only 12 are considered to be dioxin-like (Van den Berg et al. 1998).

For the purpose of assessing the human health risk from exposure to mixtures of PCDDs, PCDFs and dioxin-like PCBs, the concept of toxic equivalency factors (TEFs) has been developed. Over the years a number of different systems have been used. The dominating system during the nineties for PCDDs and PCDFs was the international system (I-TEF) that was developed by a NATO-working group in the late eighties. This system replaced more or less the German UBA-system from 1985, the Nordic system from 1988 as well as older systems developed by US-EPA. Recently, a new system was agreed upon at a WHO Consultation in 1997 (WHO-TEF) as published by Van den Berg et al. (1998).

WHO only assigned TEFs for compounds that:
a) Show a structural relationship to the PCDDs and PCDFs
b) Bind to the aryl hydrocarbon (Ah) receptor
c) Elicit Ah receptor-mediated biochemical and toxic responses
d) Are persistent and accumulate in the food chain.

A TEF for a compound is determined as the toxicity of the compound relative to the toxicity of 2,3,7,8-tetraCDD based on available *in vitro* and *in vivo* data (Van den Berg et al. 1998). In contrast to previous evaluations, WHO besides establishing TEFs for humans/mammals also provided TEFs for fish and birds. For humans/mammals the differences between the new WHO system and previous systems were that 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD) was considered as toxic as 2,3,7,8-TCDD and assigned a TEF of 1, that octachlorodibenzo-*p*-dioxins (OCDD) and octachlorodibenfuran (OCDF) were assigned a TEF value 10 times smaller than previously, and that dioxin-like PCBs were included in the scheme (Van den Berg et al. 1998; SCOOP 2000). *Although a few TEF values for individual PCDDs and PCDFs differ from the previously used I-TEFs, there*

are no significant differences in the TEQs calculated for PCDDs and PCDFs.

The majority of studies assessing the combined effects of PCDD, PCDF and dioxin-like PCB congeners in complex mixtures have supported the hypothesis that the toxic effects of combinations of congeners follow dose additivity. Therefore, the concentrations and TEFs of individual congeners in a mixture may be converted into a toxic equivalent (TEQ) concentration by multiplying the analytically determined amounts of each congener by the corresponding TEF and summing the contribution from each congener using the following equation:

$$TEQ = \sum (PCDD_i \times TEF_i) + \sum (PCDF_i \times TEF_i) + \sum (PCB_i \times TEF_i)$$

(Van den Berg et al. 1998, WHO 2000)

This paper only deals with the group of PCDDs, PCDFs and dioxin-like PCBs for which a TEF has been assigned.

1.2 Physical/chemical properties

Table 1 lists the compounds for which WHO has assigned a TEF value as well as some physical and chemical properties of these compounds.

Description:

a) PCDDs exist as colourless solids or crystals in the pure state.
b) PCDFs exist as colourless solids or crystals in the pure state.
c) Commercial PCB mixtures are light yellow or dark yellow in colour. They do not crystallise, even at low temperatures, but turn into solid resins.

Purity:

c) Commercial PCB mixtures contain PCDFs at levels ranging from a few mg/kg up to 40 mg/kg.

Melting point:

a) 240-326 °C
b) 196-269 °C
c) 173-202 °C

See table 1

Boiling point: -

Density: -

Vapour pressure: See table 1. In general all the PCDDs, PCDFs and PCBs have very low vapour pressure, which decreases with increasing numbers of chlorine atoms in the molecule.

Concentration of saturated vapours: -

Vapour density: c) PCBs form vapours heavier than air.

Conversion factor: -

Flash point: c) PCBs are, in practice, fire resistant, with

rather high flash points.

Flammable limits:	-
Autoignition temp.:	-
Solubility:	See table 1. In general all the PCDDs, PCDFs and PCBs are lipophilic and have a very low water solubility which decreases with increasing numbers of chlorine atoms in the molecule.
$logP_{octanol/water}$:	See table 1.
Henry's constant:	See table 1.
pK_a-value:	-
Stability:	**c**) PCBs are chemically very stable under normal conditions; however, when heated, other toxic compounds, such as PCDFs, can be produced.
Incompatibilities:	-
Odour threshold, air:	-
References:	ATSDR (1997), ATSDR (1998), IARC (1997), IPCS (1993), Van den Berg et al. (1998)

Table 1. CAS-no., WHO-TEFs and physical/chemical properties (when available) for compounds that have been assigned a TEF value by WHO

Congener	CAS-no.	TEF for human and mammal risk assessment	Melting point (°C)	Water solubility (g/L) at about 25 °C	Vapour pressure (Pa) at 25 °C	Henry´s constant (Pa x m^3/mol)	$logP_{octanol/water}$
PCDDs							
2,3,7,8-tetraCDD	1746-01-6	1	305-306	1.93×10^{-6}	2.0×10^{-7}	3.34	6.80
1,2,3,7,8-pentaCDD	40321-76-4	1	240-241		5.8×10^{-8}		6.64
1,2,3,4,7,8-hexaCDD	39227-28-6	0.1	273-275	4.42×10^{-9}	5.1×10^{-9}	1.08	7.80
1,2,3,6,7,8-hexaCDD	57653-85-7	0.1	285-286		4.8×10^{-9}		
1,2,3,7,8,9-hexaCDD	19408-74-3	0.1	243-244		6.5×10^{-9}		
1,2,3,4,6,7,8-heptaCDD	35822-46-9	0.01	264-265	2.40×10^{-9}	7.5×10^{-10}	1.27	8.00
OctaCDD	3268-87-9	0.0001	325-326	0.74×10^{-10}	1.1×10^{-10}	0.68	8.20
PCDFs							
2,3,7,8-tetraCDF	51207-31-9	0.1	227-228	4.19×10^{-7}	2×10^{-6}	1.5	6.53
1,2,3,7,8-pentaCDF	57117-41-6	0.05	225-227		2.3×10^{-7}		6.79
2,3,4,7,8-pentaCDF	57117-31-4	0.5	196-196.5	2.36×10^{-7}	3.5×10^{-7}	0.5	6.92
1,2,3,4,7,8-hexaCDF	70648-26-9	0.1	225.5-226.5	8.25×10^{-9}	3.2×10^{-8}	1.43	
1,2,3,6,7,8-hexaCDF	57117-44-9	0.1	232-234	1.77×10^{-8}	2.9×10^{-8}	0.6	
1,2,3,7,8,9-hexaCDF	72918-21-9	0.1	246-249		2.4×10^{-8}		
2,3,4,6,7,8-hexaCDF	60851-34-5	0.1	239-240		2.6×10^{-8}		
1,2,3,4,6,7,8-heptaCDF	67562-39-4	0.01	236-237	1.35×10^{-9}	4.7×10^{-9}	1.4	7.92
1,2,3,4,7,8,9-heptaCDF	55673-89-7	0.01	221-223		6.2×10^{-9}		
OctaCDF	39001-02-0	0.0001	258-269	1.16×10^{-9}	5×10^{-10}	0.2	8.78
Non-ortho PCBs							
3,3',4,4'-tetraCB (77)	32598-13-3	0.0001	173	1.75×10^{-4}	3.1×10^{-4}	4.4 - 9.5	6.04-6.63
3,4,4',5-tetraCB (81)	70362-50-4	0.0001					
3,3',4,4',5-pentaCB (126)	57465-28-8	0.1					
3,3',4,4',5,5'-hexaCB (169)	32774-16-6	0.01	201-202	3.6×10^{-5} - 1.2×10^{-2}	5.3×10^{-5}	1.5 - 6.0	7.41
Mono-ortho PCBs							
2,3,3',4,4'-pentaCB (105)	32598-14-4	0.0001					
2,3,4,4',5-pentaCB (114)	74472-37-0	0.0005					
2,3',4,4',5-pentaCB (118)	31508-00-6	0.0001					
2',3,4,4',5-pentaCB (123)	65510-44-3	0.0001					
2,3,3',4,4',5-hexaCB (156)	38380-08-4	0.0005					
2,3,3',4,4',5'-hexaCB (157)	69782-90-7	0.0005					
2,3',4,4',5,5'-hexaCB (167)	52663-72-6	0.00001					
2,3,3',4,4',5,5'-heptaCB (189)	39635-31-9	0.0001					

1.3 Production and use

Since the 1930s, PCBs have been produced commercially by chlorination of biphenyl with anhydrous chlorine under heated reaction conditions and in the presence of a catalyst (e.g. iron-chloride). The result is always a mixture of different congeners and impurities (mainly PCDFs) (ATSDR 1997, IPCS 1993).

Until the middle of the 1970es, PCBs have been used extensively (the total world production of PCBs was still in excess of 1 million tonnes in 1980) in closed as well as open systems.

Examples of use in closed systems:

a) In electric equipment such as capacitors and transformers
b) As fire-resistant liquid in heat transfer and hydraulic systems

Examples of use in open systems:

a) Plasticizers
b) Surface coatings
c) Inks
d) Adhesives
e) Flame retardants
f) Pesticide extenders
g) Paints
h) Microencapsulating of dyes
i) Carbonless duplicating paper
j) Immersion oils for microscopes
k) Catalyst in the chemical industry
l) Casting waxes in the iron/steel industry
m) Cutting and lubricating oils

Today the PCBs have been replaced in many products. (ATSDR 1997; IPCS 1993). Since 1986 no new products containing PCBs have been allowed in Denmark. Continued use of old products has only been allowed in closed systems (Sundhedsstyrelsen 1999).

PCDDs and PCDFs are not produced intentionally except on a small scale for use in chemical and toxicological research. They are formed as by-products, e.g. during the chemical manufacture of a number of chlorinated compounds, during chlorine bleaching of paper pulp and various other industrial processes in the presence of chlorine, and during incomplete combustion processes, industrial as well as natural (ATSDR 1998, IARC 1997). The formation of dioxins in Denmark in 1998-99 has been estimated at 90-830 g I-TEQ per year (MST 2000).

PCDD is conventionally produced for research by condensation of polychlorophenols or by direct halogenation of the parent dibenzo-*p*-dioxin (ATSDR 1998).

1.4 Environmental occurrence

Due to their high persistency, PCDDs, PCDFs and PCBs are present in the environment all over the world. The composition of the mixtures of congeners found in the environment differs from the technical mixtures

originally released into the environment because of differences in the rate of degradation of the individual congeners (Van den Berg 1998).

Estimates of the substance flow of dioxins in Denmark in 1998-99 have been carried out (MST 2000). It was estimated that between 90 – 830 g I-TEQ was formed per year from waste incineration, energy production, and industrial or other activities. Significant amounts were emitted to air bound on particles from where most of it was deposited in soil and water. Other significant amounts were estimated to remain in residues, such as ash and sludge, and subsequently deposited.

1.4.1 Air

Globally, PCBs have been found in ambient air at concentrations from 0.002 to 15 ng/m^3 of total-PCB of which dioxin-like PCBs are thought to only constitute a minor fraction. In industrial areas, levels may be higher (up to μg/m^3) (IPCS 1993). No Danish studies of the concentration of PCDDs and PCDFs in the ambient air have been conducted, however measurements are being conducted at present and the first indications concerning the PCDD and PCDF levels in ambient air in Denmark may be available in the near future. Typical concentrations measured in Europe are from 0.01 pg I-TEQ/m^3 in rural areas to 0.1-0.4 pg I-TEQ/m^3 in urban areas. Emission of PCDDs and PCDFs in Denmark seems to decrease (MST 1997). The total emission to the atmosphere in 1998-99 from all known sources was estimated to be 19-170 g I-TEQ/year in Denmark. The largest atmospheric source was incineration of municipal solid waste (MST 2000).

1.4.2 Water

Drinking water contains less than 1 ng total-PCB/litre (IPCS 1993). No Danish studies of the concentration of PCDDs and PCDFs in the water have been published. The low water solubility results in very low background levels in the water phase and a tendency to accumulate in sediments at about 1 ng I-TEQ/g dry weight (MST 1997). The total emission to the water in 1998-99 from all known sources was estimated to be 0.3-1.4 g I-TEQ/year in Denmark (MST 2000).

1.4.3 Soil

Soil and sediments in different areas contain levels of total-PCB ranging from <0.01 up to 2.0 mg/kg (IPCS 1993). In Europe the concentration of PCDDs and PCDFs in soils have been measured to correspond to 0.3 - 112 ng I-TEQ/kg dry weight (MST 1997). For Denmark in 1998-99, it was estimated that 16-160 g I-TEQ/year was deposited from air to soil. An additional 1.3-54 g I-TEQ/year was estimated to result from direct contamination of soil from various sources (MST 2000). At present further measurements are conducted in order to obtain a better basis for assessing the PCDD and PCDF levels in Danish soil.

Vikelsøe (2002) examined PCDDs and PCDFs in Danish soils collected in the fall of 2001 from various industrial/urban (exposed) locations, as well as rural (reference) locations. In preserved topsoils almost all PCDD/PCDF was found in the upper 0-10 cm. The exposed samples contained from 0.25 – 3 ng I-TEQ per kg dry weight, with only three samples being above 1 ng I-TEQ per kg dry weight. The mean concentration of the reference samples

was 0.67 ng I-TEQ per kg dry weight. The author concluded that the study did not in general indicate significantly elevated levels near larger industrial/urban centres or point sources, although the contamination in the Copenhagen area was significantly higher than at its reference points.

1.4.4 Food

Fatty fish (up to 10 pg I-TEQ/g product), milk and dairy products (up to 3 pg I-TEQ/g fat) and meat and meat products (up to 3 pg I-TEQ/g fat) are the main foodstuffs containing the PCDD and PCDF congeners. A decreasing trend in the concentration of PCDDs and PCDFs in foods has been reported for a few countries in Europe. The dioxin-like PCBs seem to contribute to the TEQ with one to two times the TEQ contribution of PCDDs and PCDFs (SCOOP 2000, SCF 2000).

1.5 Environmental fate

1.5.1 Air

Some PCDDs, PCDFs and PCBs will be present in the vapour phase. At lower temperatures and with an increasing number of chlorine atoms in the molecule, the congeners will mostly be attached to particles and aerosols. They are rather stable in the atmosphere. In general, persistence of congeners increases with the degree of chlorination. Photolysis may be the most important degradation process. However, the majority of PCDDs, PCDFs and PCBs are deposited to soil or water (IPCS 1993, MST 1997).

1.5.2 Water

PCDDs, PCDFs and PCBs have a tendency to accumulate in sediments because of their low water solubility (IPCS 1993, MST 1997). Preliminary estimates of degradation half-lives in nature indicate half-lives in water and sediments ranging from around 30 years to around 200 years. Biological reactions in sediments are believed to cause a dechlorination of higher chlorinated dioxins like octaCDD thereby transforming these into 2,3,7,8-tetraCDD and lower chlorinated dioxins (MST 2000).

1.5.3 Soil

PCDDs, PCDFs and PCBs binds to soil particles and are considered rather immobile in soil and will mainly concentrate in the topsoil layer unless they are mechanically spread. Biodegradation in soil is negligible. Some photolytic degradation in the surface layer may occur. However, the half-life of for instance 2,3,7,8-tetraCDD in soil has been estimated to be more than 10 years. (IPCS 1993, MST 1997).

1.5.4 Bioaccumulation

Since dioxins and dioxin-like PCBs are very lipophilic and extremely resistant towards chemical and biological degradation processes, they persist in the environment and accumulate in food chains (SCF 2000). Bioconcentrations factors of 200-70000 have been measured for various aquatic organisms (IPCS 1993, MST 1997).

PCDDs, PCDFs and PCBs may be absorbed by the plant roots, which accumulate more than the stems and foliage but the bioconcentration factors are low (IPCS 1993, MST 1997).

1.6 Human exposure

More than 90 % of the general human exposure to dioxins and dioxin-like PCBs is estimated to occur through the diet. Data from the EU SCOOP report indicate that the average daily intake of PCDDs and PCDFs in various North European countries is in the range of 0.4-1.5 pg WHO TEQ/kg bw per day (for the period after 1995). When the dioxin-like PCBs are included an additional intake of 0.8-1.8 pg WHO TEQ/kg bw per day should be considered. Although no recent thorough survey of the dietary intake of PCDDs, PCDFs and dioxin-like PCBs are yet available for Denmark a preliminary estimate indicates a similar situation. Within the general population, some subpopulations may be exposed to higher amounts of dioxins and dioxin-like PCBs as a result of particular dietary habits e.g. breastfeed infants and frequent consumers of high amounts of contaminated fish (SCOOP 2000, SCF 2000).

Within the past few years, a substantial reduction in the intake of PCDDs, PCDFs and PCBs has occurred reflecting the regulatory measures taken to reduce emissions in the late 1980es (WHO 2000, SCF 2000).

Older data has been used to estimate a total exposure to PCDDs and PCDFs of about 200 pg I-TEQ/day in Danish adults. In the same publication an intake in children of 4 pg I-TEQ/day through soil ingestion was estimated (MST 1997).

Due to the accumulation of PCDDs, PCDFs and PCBs in human fat, human milk can contain high concentrations of these compounds. Approximately 30 pg WHO-TEQ/g fat would be a reasonable estimate for the average concentration of PCDDs, PCDFs and PCBs in humans in the Western European countries (SCOOP 2000), including Denmark (Personal information from the Danish Food Administration). This means that the suckling baby may ingest as much as 180 pg WHO-TEQ/kg bw/day, assuming an intake of 6 g fat/kg bw/day from mothers milk.

2 Toxicokinetics

The three main factors that governs the toxicokinetics of individual PCDD, PCDF and PCB congeners are:

a) The lipophilicity
b) The degree of binding to cytochrome P4501A2 (CYP1A2) in the liver
c) The rate of metabolism of the compound

The lipophilicity controls the rate and extent of absorption, tissue distribution and passive elimination. Binding to CYP1A2 results in hepatic sequestration of the congeners. The chlorine substitution pattern determines the degree of this binding as well as the metabolic rate (Van den Berg 1998).

2.1 Absorption, distribution

Molecular size and lipid solubility of a congener are the rate limiting factors for the absorption from the gastrointestinal tract. Congeners having 4, 5, or 6 chlorine atoms are well absorbed (50-90 % depending on the vehicle) while hepta-and octa chlorinated congeners are absorbed to a lesser extent. The absorption rate of 2,3,7,8-tetraCDD given by a bolus dose in corn oil to pregnant rats was about 60% (SCF 2000). The uptake by dermal permeation and pulmonary absorption is considered to be more limited than the uptake after oral ingestion (IARC 1997). For combustion particles, such as fly ash, being the major source to PCDDs and PCDFs in ambient air, a bioavailability of 5 – 20 % has been proposed, whereas the bioavailability after dermal contact is probably less than 1 % (IARC 1997).

Due to their high lipophilicity and resistance to biotransformation the congeners accumulate in the body, mainly in adipose tissue and liver. After absorption from the gastrointestinal tract, 2,3,7,8-tetraCDD enters the lymph in the form of chylomicrons and is then cleared from the blood within 1 h. Cleared 2,3,7,8-tetraCDD appears mainly (74-81% of an administered dose) in the liver and adipose tissue. After clearance of chylomicrons, dioxin-like compounds remain mainly in serum lipoproteins and some are bound to serum proteins. Only small amounts of highly chlorinated congeners will accumulate after exposure to a single high dose because of limited absorption. However, the same congener may accumulate significantly in tissues and lead to a biological response if it is administered in relatively low doses over a longer period of time (Van den Berg 1998). Activation of the Ah receptor by binding of congeners to it increases the amount of CYP1A2 (IARC 1997). A number of highly toxic congeners such as 2,3,4,7,8-pentaCDF, 2,3,7,8-tetraCDD, and PCB 126 bind very tightly to CYP1A2 and subsequently concentrate in the liver in many rodent species, even at low dose levels. The hepatic accumulation of PCBs decreases dramatically with the addition of one chlorine atom in ortho position. The liver/adipose tissue distribution can thus vary significantly between different congeners (Van den Berg 1998). The liver/adipose tissue distribution also varies between different species. In general, humans have greater fat stores than rats and will accumulate more of the very lipophilic congeners in adipose tissue and less in target tissues such as the liver. In humans, the liver concentration of congeners is about 1/10 of the level in adipose tissue. In rats, the tissue distribution is dose-dependent with about the same concentration in liver and

fat at a single dose of 1 ng/kg bw but a five times higher concentration in the liver than in fat at a single dose of 1 µg/kg bw (Neubert 1997/98). The structure-activity relationship for binding to the Ah receptor and to CYP1A2 is not identical (Van den Berg 1998). Congeners can pass the placenta of pregnant animals and humans (IARC 1997, IPCS 1993).

Co-administration to rats of PCDDs, PCDFs and dioxin-like PCBs with the non dioxin-like congener PCB 153 resulted in modulation of the hepatic disposition and elimination. To what extent these toxicokinetic interactions are also relevant at low-level environmental exposure is still unknown (Van den Berg 1998).

2.2 Elimination

2.2.1 Metabolism

Many PCDDs, PCDFs and PCBs are very resistant to metabolism and therefore bioaccumulate. The major determinant for metabolism of the congeners is the presence of two adjacent, unsubstituted carbon atoms on the lateral positions. These positions are preferentially oxidised by the cytochrome P450 system resulting in more polar metabolites. PCDFs are more susceptible to biochemical degradation than PCDDs because of the stress on the furan ring. In addition, the positions (4 and 6) adjacent to the oxygen bridge in the PCDFs are more sensitive to metabolic attack than those in the PCDDs (Van den Berg 1998).

2.2.2 Excretion

In almost all laboratory species, which have been studied elimination takes place through bile and faeces in the form of hydroxylated or conjugated metabolites. Non-absorbed congeners are excreted with the faeces (IARC 1997).

2.2.3 Half-life

In rats, the half-life of 2,3,7,8-tetraCDD ranged from 17 to 31 days. In rhesus monkeys, an average half-life of about 400 days has been determined for 2,3,7,8-tetraCDD. However, in humans the half-life of this congener has been reported to range from 5.5 to 11 years. Other congeners have been estimated to have half-lives between 3 and 50 years in humans. The apparent half-life is not absolute but may vary with dose, body composition, age and sex (IARC 1997, WHO 2000).

2.2.4 Body burden and content in human milk

Due to the resistance against biotransformation and the consequently long half-life in humans, PCDDs, PCDFs and PCBs accumulate in human fat tissues. For the period 1995-1999 the national average concentrations of PCDDs and PCDFs in human milk in Europe ranged between 8 and 16 pg I-TEQ/g fat. For dioxin-like PCBs the contribution to the TEQ seem to be from one to three times the TEQ for PCDDs and PCDFs (SCOOP 2000). In a recent, as yet unpublished study, 16 samples of Danish human milk were analysed for PCDDs, PCDFs and dioxin-like PCB. Total concentrations

ranged from 14.8 – 43.6 ng WHO-TEQ/kg fat (Personal information from the Danish Food Administration, 2002).

2.3 Mechanisms of action

A broad variety of data primarily on 2,3,7,8-tetraCDD but also on other dioxin-like compounds in many experimental models using multiple species, including humans, have shown that binding to the intracellular aryl hydrocarbon (Ah) receptor is important in mediating the biochemical and toxic effects of PCDDs, PCDFs and dioxin-like PCBs. However, the precise chain of molecular events by which this ligand-activated receptor elicits these effects is not fully understood yet (SCF 2000, WHO 2000).

The Ah receptor binding affinity of the individual congeners is dependent on the extent and pattern of chlorination. In general, the congeners most similar to 2,3,7,8-tetraCDD have the highest binding affinity and thus show the strongest toxicity (IARC 1997, IPCS 1993).

The exact physiological role of the Ah receptor is not known but it may be involved in the embryonic development. Studies using Ah-receptor-deficient mice have documented a spectrum of pathological lesions and indicated a role of the receptor in the normal growth and development of the liver and the immune system (SCF 2000). When xenobiotics bind to and activate the receptor, it functions as a transcription factor that recognises DNA of target genes and enhances the transcription of them. The Ah receptor may also modulate biochemical and cellular responses via non-DNA dependent mechanisms. Resulting biochemical responses are:

a) Induction of drug-metabolising enzymes such as cytochrome P4501A1, cytochrome P4501A2, cytochrome P4501B1, aldehyde-3-dehydrogenase, glutathione S-transferase (GST), uridine diphosphate glucuronosyltransferase (UDP-GT), Nad(P)H:quinone oxidoreductase and prostaglandin endoperoxide H synthase-2.
b) Modulation of growth factors, growth factor receptors, transcription factors, lymphokines and related factors.
c) Modulation of thyroid hormones, vitamin A and retinoids.
d) Modulation of protein phosphorylation.
e) Modulation of biochemical responses associated with glucose metabolism and transport.
f) Modulation of oestrogenic responses.
g) Induction of oxidative stress in various tissues, probably related to the induction of cytochromes, resulting in for instance enhancement of lipid peroxidation.
h) Modulation of cell cycle regulation and apoptosis.

These biochemical responses, some of which may be adaptive to dioxin exposure, may or may not lead to toxic effects at higher exposure levels (IARC 1997).

3 Human toxicity

3.1 General toxicity

3.1.1 Cardiovascular effects

In a number of industrial cohorts exposed to PCDD and PCDF contaminated productions, the mortality from all diseases of the circulatory system was similar to the mortality in the general population. However in German chemical workers, mortality from cardiovascular disease was positively related to estimated 2,3,7,8-tetraCDD levels and significantly related to estimated total TEQ concentrations above 39 ng/kg lipid. Studies of Viet Nam veterans that had been exposed to widely used herbicides contaminated with 2,3,7,8-tetraCDD and other dioxins and of people from Seveso that had been exposed to 2,3,7,8-tetraCDD after an industrial accident where several kilograms was released into the air have also revealed increases in mortality from cardiovascular disease. In a few studies, ischaemic heart disease, increased blood pressure and arrhythmias have been associated with high exposure to 2,3,7,8-tetraCDD (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

3.1.2 Dermal effects

Chloracne is the most widely recognised dermal effect of exposure to PCDDs, PCDFs and dioxin-like PCBs. It occurs shortly after acute or chronic exposure to a variety of chlorinated aromatic compounds by skin contact, ingestion or inhalation. It has occurred with or without other effects in at least a few workers in all reported accidents at trichlorophenol (TCP) production facilities and among individuals involved in production of 2,3,7,8-tetraCDD-contaminated products. Chloracne was also noted in the Seveso cohort (mostly children) and among residents in Japan (Yusho) and Taiwan (Yu-Cheng) who had consumed rice oil contaminated with a complex mixture of PCDDs, PCDFs, dioxin like PCBs, non-dioxin like PCBs and polychlorinated quaterphenyls and -terphenyls. Among Seveso residents, the chloracne resolved in all but one person within 7 years despite of high serum 2,3,7,8-tetraCDD levels. However, in TCP workers the mean duration of chloracne has been reported to be 26 years. A threshold level above which chloracne occurs has not been established but a study has estimated that some TCP production workers with diagnosed chloracne had adipose levels of 2,3,7,8-tetraCDD greater than 200 ng/kg lipid (ATSDR 1997, IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

Hyperpigmentation have been reported among workers exposed to 2,3,7,8-tetraCDD and among residents in Japan and Taiwan exposed to contaminated rice oil (IARC 1997, IPCS 1993, WHO 2000).

3.1.3 Diabetes

Marginally elevated mean fasting glucose levels were found in a study with German workers exposed to 2,3,7,8-tetraCDD. Among US production workers, the overall prevalence of diabetes mellitus was not significantly

different from controls. However, an increased prevalence was found in workers with serum 2,3,7,8-tetraCDD concentrations in excess of 1500 ng/kg lipid. An increased risk for elevated fasting glucose levels and diabetes was found in the Viet Nam veterans who had levels of 2,3,7,8-tetraCDD above 94 ng/kg lipid (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

3.1.4 Gastrointestinal effects

Transient nausea, vomiting and abdominal pain has been observed in residents in Japan and Taiwan exposed to contaminated rice oil as well as in some chemical workers (IARC 1997).

3.1.5 Hepatic effects

Transient elevations in liver enzyme (γ-glutamyltransferase, aspartate and alanine aminotransferase) and D-glucaric acid levels in both children and adults have been observed among TCP production workers as well as among residents in areas of industrial accidents with dioxins or dioxin-like compounds (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

Increased concentrations of urinary D-glucaric acid were found in adults and children in Seveso in 1976. However, by 1981 the concentrations were within the normal range (JECFA 2002).

Evidence of alterations in porphyrin metabolism among populations exposed to 2,3,7,8-tetraCDD is inconsistent (IARC 1997, SCF 2000, WHO 2000, JECFA 2002)).

A number of case reports and epidemiological studies have described increases in level of serum lipid fractions, particularly total cholesterol and triglycerides in people exposed to PCDDs, PCDFs and PCBs in high doses. However, the majority of epidemiological studies of workers and community residents have reported no significant increases in total cholesterol or triglycerides levels among exposed populations compared with controls (IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

In Japan, no definite signs of liver enlargements or disorders but slight rises in liver enzymes were detected in people exposed to contaminated rice oil. A liver sample from a patient showed an increase in the amount of smooth endoplasmatic reticulum. Mortality from chronic liver disease and cirrhosis was elevated in men (IARC 1997, IPCS 1993, WHO 2000).

3.1.6 Immunological effects

Most epidemiological studies have not found a clear relationship between exposure to PCDDs, PCDFs and PCBs and impaired immunological status. However, alterations in the level and function of different antibodies, lymphocytes and complement proteins have been measured in some of the studies (IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

3.1.7 Neurological effects

Workers exposed to PCBs have complained of headache, dizziness, depression, fatigue, nervousness, and sleeping and memory problems. Transient neurological symptoms were reported in some of the Japanese and Taiwanese people exposed to contaminated rice oil. The symptoms in Japan included limb paraesthesia and spasms, weakness, headaches and fatigue. Viet Nam veterans who had serum levels of 2,3,7,8-tetraCDD above 33.3 ng/kg lipid tended to have a higher proportion of individuals with abnormal coordination than comparisons. A lot of other studies has not shown any relationship between exposure to 2,3,7,8-tetraCDD and neurological effects (ATSDR 1997, IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002)).

3.1.8 Ocular effects

Ophthalmological changes (swelling and hypersecretion of the glands in the eye lid and pigmentation of the conjunctiva), which in some cases appeared to persist 15 years after exposure ended, were observed in most of the exposed people in Japan and Taiwan (IARC 1997, IPCS 1993).

3.1.9 Respiratory effects

Conflicting evidence exist from epidemiological studies regarding an association between human exposure to 2,3,7,8-tetraCDD and chronic effects on the respiratory system. In Japan, chronic bronchitis and respiratory infections were seen and still remained in some individuals 14 years after exposure ended (IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

3.1.10 Thyroid effects

Most studies of exposed humans have found parameters of thyroid function (thyroid-stimulating hormone (TSH), thyroxine (T4), thyroid -binding globulin (TBG)) within normal range although in some studies their levels were related to 2,3,7,8-tetraCDD levels (IARC 1997, SCF 2000, WHO 2000).

3.2 Toxicity to reproduction

Most studies on reproductive effects of PCDDs in humans have concerned paternal exposure of workers or Viet Nam veterans. Some studies have shown alterations in sex hormone levels (elevated serum levels of luteinizing hormone and follicle-stimulating hormone and a decreased level of testosterone) and sperm characteristics (lower concentration, percentage of motile cells and percentage of morphological normal cells) after PCDD exposure. Discordant results exist for an increase in the risk of spontaneous abortions among the wives of the exposed men. In most of the studies an elevation in birth defects were not detected. (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

An alteration of the sex ratio was observed between 1977 and 1984 in children born to parents highly exposed to 2,3,7,8-tetraCDD after the industrial accident in Seveso in 1976. Paternal serum lipid concentrations of 2,3,7,8-tetraCDD higher than 118 ng/kg lipid at the time of conception were

associated with the birth of significantly more girls than boys (twice as many). The decreased male/female sex ratio might already be apparent at serum concentration between 15 and 80 ng/kg lipid in the father. During 1985 – 1994 the sex ratio reverted to normal. An explanation of this phenomenon has not been offered but a possible role of hormonal disruption cannot be ruled out. However, no changes in sex ratio have been observed in other studies including a study of the Taiwanese cohort having very heavy maternal body burdens (estimated levels at 2 – 3000 ng TEQ/kg bw) (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

Two US birth cohorts with measured background exposure to total-PCBs have been followed since 1980, and 2 Dutch birth cohorts with measured background levels of PCBs, PCDDs and PCDFs have been followed since 1990. Mothers in both US cohorts were presumably also exposed to other chlorinated pesticides and heavy metals. Some data are available on Japanese and Taiwanese children exposed transplacentally to a complex mixture of polychlorinated compounds. In all the studies of infants and children, effects were primarily associated with *in utero*, rather than lactational exposure. Analysis of placental samples collected from mothers affected in Taiwan 5 years following exposure revealed 610-9010 ng TEQ/kg lipid. Average concentrations seen in the US placentas were 8.4-17.6 ng TEQ/kg lipid. In Japan, the lowest intake estimated to result in minimal symptoms was 28 ng TEQ/kg bw/day for 135 days. (IARC 1997, IPCS 1993, SCF 2000, WHO 2000).

In Japan and Taiwan effects on the children included ectodermal defects, global persistent developmental delays, low birth-weight, mild persistent behaviour disorders, decrease in penile length at puberty, reduced height among girls at puberty and hearing loss. Neurodevelopmental delays and neurobehavioral effects including neonatal hypotonia also occurred in the three largest cohorts, two in the US and one in The Netherlands, although the age at which the effects occurred and the tests used to detect them were not the same. In the two US cohorts, the observed neurobehavioral effects were limited to the most highly PCB exposed infants. (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

Thyroid hormone levels were evaluated in the two cohorts in The Netherlands with similar exposure to PCDDs/DFs and total PCBs. In utero exposure to total TEQs, as measured in mother's milk, may have influenced thyroid hormone status (TT4, TSH) in infants up to 3 months of age. (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

3.3 Mutagenic and genotoxic effects

No statistically significant difference was found in the frequencies of chromosomal aberrations or sister chromatid exchanges in peripheral lymphocytes in male workers exposed to 2,3,7,8-tetraCDD in a German accident and in Taiwanese women exposed to PCBs and PCDFs in contaminated rice oil compared to unexposed controls. However, exposure to PCBs and PCDFs enhanced the sensitivity of lymphocytes to sister chromatid exchanges induced by α-naphthoflavone. (IARC 1997). A slight increase in sister chromatid exchanges and chromosomal aberrations in lymphocytes have been described in workers involved in the manufacture of PCBs and in workers exposed to PCBs following a fire in an electric station. However, in both studies exposure to chemicals other than PCBs may have occurred (ATSDR 1997). In the Seveso accident, the frequency of aberrant cells in maternal peripheral lymphocytes, and placental and umbilical cord

tissues was not increased in women exposed to 2,3,7,8-tetraCDD compared to unexposed controls. However, significant increases in aberrations were noted in foetal tissues. No DNA-adducts were detected in placentas from non-smoking Taiwanese women exposed to PCBs and PCDFs in contaminated rice oil (IARC 1997).

3.4 Carcinogenic effects

In most of the human epidemiological studies examining the carcinogenicity of PCDDs, PCDFs and PCBs, workers were exposed to mixtures of PCDDs including 2,3,7,8-tetraCDD, as contaminants of phenoxy herbicides and chlorophenols. At the time of exposure, the blood lipid level of 2,3,7,8-tetraCDD was estimated to be 2000 ng/kg lipid (mean) (up to 32000 ng/kg) in a cohort involving 12 industrial plants in the USA, 1434 ng/kg lipid (mean) (range 301-3683 ng/kg) among Dutch workers involved in the clean up of a reactor explosion, 1008 ng/kg lipid (mean) in a group of German workers with severe chloracne after an accident at a TCP production unit, and up to 2252 ng/kg lipid in another German cohort from Boehringer. After an industrial accident in Seveso the blood lipid level of 2,3,7,8-tetraCDD in inhabitants was estimated to be 389 ng/kg lipid (median) in zone A (≥50 μg 2,3,7,8-tetraCDD/m^3 soil) and 78 ng/kg lipid (median) in zone B (≥5 and ≤ 50 μg 2,3,7,8-tetraCDD/m^3 soil). The upper 75th percentile in zone A was about 2000 ng/kg lipid. Contaminated rice oil was consumed in Japan and Taiwan. For the Japanese oil, the daily intake was estimated at 0.33 mg PCBs/kg bw/day and 0.002 mg PCDFs/kg bw/day for about a month. For the Taiwanese oil, the daily intake was estimated at 0.06 mg PCBs/kg bw/day and 0.0002 mg PCDFs/kg bw/day for 10 months (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

Low excess risks on the order of 40% for all neoplasms combined were seen in the occupational cohort studies in which the exposure assessment was adequate. Tests for trends to increasing excess risks for all neoplasms combined with increasing intensity of exposure were statistically significant. Thus, the German cohort (with workers with severe chloracne) evaluated dose-response both for estimated exposure to 2,3,7,8-tetraCDD and to PCDD/PCDFs using TEQ and identified a positive trend in both analyses. Risks for cancers at specific sites were increased in some of the studies, but the results are not consistent between studies and no single cancer site seemed to predominate. In Seveso, all-cancer mortality did not differ significantly from that expected in any of the contaminated zones, although excess risk were seen for specific cancers in the most heavily contaminated zones, but the numbers of cases are small. Follow-up for the Seveso cohort was shorter than for the occupational cohorts. In most of these studies excess risks were observed for soft tissue sarcoma, lung cancer, non-Hodgkin lymphoma and digestive tract cancers. Statistically significant excess risks were observed in individual cohorts for a variety of other cancers including multiple myeloma, oral cavity cancer, kidney cancer, leukemia and breast cancer in women. In Japan there was an excess risk for liver cancer at 22 years of follow-up. No excess cancer risk was observed in Taiwan at 12 years of follow-up (IARC 1997, IPCS 1993, SCF 2000, WHO 2000, JECFA 2002).

Although the excess cancer risk at the highest exposure was statistically significant in these studies, the results must be evaluated with caution, as the overall risks are not high and the strongest evidence is for industrial populations with two to three orders of magnitude greater exposure than the

general population. The industrial populations also had heavy exposure to other chemicals.

JECFA (2002) explored the calculation of a "benchmark dose" (e.g., the ED_{01}, the dose estimated to result in a 1% increase in cancer mortality), on the basis of a meta-analysis of data from three industrial cohorts with well-documented exposure to TCDD. A statistically significant linear trend in risk with exposure was observed, which persisted even after exclusion of groups with the highest exposure. The ED_{01} ranged quite widely and strongly depended on the assumptions made. JECFA (2002) estimated an ED_{01} = 41 pg/kg/day (90% CI: 22, 131). This value is best interpreted as an exposure above the current background TCDD-TEQ level that predicts to increase the risk of cancer mortality by 1% above the current level (which includes any contribution by background levels of TCDD-TEQ to background cancer mortality). JECFA also cited assessments performed by the U.S. Environmental Protection Agency (USEPA 2000) and by Steenland *et al.* (2001). In its draft health effects dioxin document, USEPA (2000) applied a linear model to the same three cohorts to predict lifetime risk of all cancer. This analysis differed in a number of ways from the analysis performed by JECFA. Based on their meta-analysis USEPA estimated an ED_{01} of 47 ppt lipid concentration and 95% lower bound of 30 ppt. This lipid concentration would correspond to a daily intake of 5.9 pg/kg bw/day (95% lower bound = 3.7 pg/kg bw/day). Steenland *et al.* (2001) estimated additional lifetime risk of cancer from TCDD exposure using two models. One model assumed that relative risk was a linear function of the log cumulative TCDD serum level, and the second assumed that the relative risk was a piece-wise linear function of (untransformed) cumulative TCDD serum level. The latter model was similar to the model applied by JECFA. This model predicted an ED_{01} = 111 ppt, which, corresponds to a daily intake of 14 pg/kg bw/day. The log-linear model studied by Steenland *et al.* (2001) was considered to be less plausible than the linear model. For example, this model predicts that the current 5 ppt background serum TCDD level is responsible for 44% of all cancers, and increasing the serum concentration another 100-fold (from 5 ppt to 500 ppt) will only cause about the same increase in risk as that predicted for the current background of 5 ppt (JECFA 2001).

4 Toxicity, animal data

4.1 General toxicity

Many of the toxicological effects following exposure are the same irrespective of the intake being acute or chronic (SCF 2000, WHO 2000). Most animal studies have been performed using 2,3,7,8-tetraCDD.

4.1.1 Biochemical effects

A number of biochemical changes (see chapter 2.3 on “Toxicological mechanism”) such as liver enzyme induction, enhanced expression of growth factors and enhanced oxidative stress have been noted in experimental animals at doses above 100 pg/kg bw per day equivalent to body burdens of 3-10 ng/kg bw (IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.2 Cardiovascular effects

High, near lethal doses of 2,3,7,8-tetraCDD alter cardiac function and morphology in several animal species (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000).

4.1.3 Dermal effects

In rhesus monkeys, a single dose of 1 μg/kg bw of 2,3,7,8-tetraCDD induced chloracne (IARC 1997). Chloracne has also been seen in cows, horses, rabbits and hairless mice (WHO 2000).

4.1.4 Endocrine effects

A target organ for 2,3,7,8-tetraCDD is the pituitary resulting in disturbances of the levels of sex steroids, corticosteroids and thyroid hormones. A decrease in the thyroid hormone T4 and an increase in TSH as well as follicular cell hyperplasia and hypertrophy have been observed in rats and mice exposed to a single dose of 2,3,7,8-tetraCDD in the μg/kg bw range or repeated exposure at lower doses. Increased adrenocorticotropin levels and alterations in corticosterone levels as well as disruptions in the normal feedback mechanisms between luteinizing hormone and the sex steroids and gonadotropin releasing hormone have been measured in rat studies (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.5 Gastrointestinal effects

A significant increase in the serum level of gastrin as well as hyperplasia of the stomach epithelia in response to toxic doses of 2,3,7,8-tetraCDD has been observed in several species. Monkeys are more sensitive than rodents to gastrointestinal effects of 2,3,7,8-tetraCDD (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.6 Hepatic effects

Liver hyperplasia, fatty infiltration, and necrosis have been observed in a number of species fed 2,3,7,8-tetraCDD in the μg/kg bw dose range. Liver toxicity is associated with increased serum transaminases and dehydrogenases, and impaired biliary clearance. Altered lipid metabolism results in elevated serum triglycerides and cholesterol, as well as decreased serum glucose levels. Accumulation of porphyrins has also been observed (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.7 Immunological effects

Thymic atrophy and suppression of humoral immunity occur at doses causing other overt signs of toxicity in multiple animal species. 2,3,7,8-TetraCDD has caused suppression of cell-mediated immunity after exposure to doses as low as 10 ng/kg bw in rats. The most sensitive immunological effect reported in mice is enhanced mortality due to influenza after exposure to a single dose of 10 ng/kg bw. However, no dose-response relationship existed. In studies in monkeys, alterations in the ratio of different subsets of T-lymphocytes have been observed at the same low doses. However, some doses lead to increases and other to decreases of a certain subset of T-cells with no clear pattern. Neonates and young animals are much more sensitive than adults to most of the immunological responses (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.8 Lethality

The acute toxicity of 2,3,7,8-tetraCDD and related PCDDs and PCDFs substituted in at least the 2, 3, 7, and 8 positions varies widely between and among species. For example, the oral LD_{50} in guinea-pigs was 0.6 μg/kg of body weight, while that in hamsters was greater than 5000 μg/kg of body weight. While data on acute toxicity are available for various commercial PCB mixtures (LD_{50} values usually greater than 100 mg/kg of body weight), there is limited data on the individual coplanar PCB congeners in mammals.

In all mammalians species tested so far, lethal doses of PCDDs and PCDFs result in a generalised delayed wasting syndrome that precedes death. It is characterised by inhibition of gluconeogenesis, reduced feed intake, and excessive loss of body weight. Although some species differences exist, other toxic effects observed after acute exposure to PCDDs include haemorrhages in a number of organs, thymic atrophy, hypertrophy/ hyperplasia of hepatic, gastrointestinal, urogenital and cutaneous epithelia, atrophy of the gonads, subcutaneous oedema and systemic haemorrhage, and reduced bone-marrow cellularity (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.9 Neurological effects

Adult animals exposed to relatively high 2,3,7,8-tetraCDD doses exhibit behavioural signs indicative of effects on the central nervous system. Minor changes in the brain neurotransmitter system, a slowing of sensory and motor conduction velocities as well as a progressive neuropathy have been

reported in rats. (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.1.10 Respiratory effects

Only few studies have examined the respiratory system in animals after oral exposure to PCDDs. Haemorrhage and hyperplasia of the bronchial epithelium (as well as at other organ sites that had mucous-secreting cells) developed in monkeys fed 2,3,7,8-tetraCDD but in rodents conflicting evidence exist. (ATSDR 1998).

4.2 Reproductive and developmental effects

PCDDs, PCDFs and PCBs are developmental and reproductive toxicants in experimental animals. Most perturbations of the reproductive system in adult animals require overtly toxic doses. In contrast, effects on the developing organism occur at doses more than 100 times lower than those required in the mother. Adverse developmental effects observed include structural malformations (cleft palate, hydronephrosis), accelerated tooth eruption and impairment of dentin and enamel formation, growth retardation, gastrointestinal haemorrhage and oedema. Sensitive targets include the developing reproductive, nervous and immune systems. Reproductive effects include delayed puberty, altered mating behaviour, decreased sperm count, and genital malformations. Effects on the nervous system include hearing deficits, changes in locomotor activity and rearing behaviour, depression of core body temperature and deficits in object learning. Immuntoxic effects include thymic and splenic atrophy, changes in cell surface markers and suppression of delayed type hypersensitivity. Perturbations of multiple hormonal systems may play a role in these events (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

4.3 Pivotal studies

WHO (2000), SCF (2000, 2001) and JECFA (2002) have listed the studies (see table 2) with the most sensitive adverse effects of 2,3,7,8-tetraCDD in animals. Except for development of endometriosis in monkeys, all of these studies show developmental or reproductive effects in the offspring of the exposed dams. However, recent studies (Rier et al. 2001) have raised a number of questions about the actual exposures that the monkeys have experienced, and the monkey studies were neither used by SCF (2001) in its up-dated risk assessment nor by JECFA (2002).

Table 2. Most sensitive adverse effects of 2,3,7,8-tetraCDD reported in animals.

Sensitive adverse effects	Exposure	Maternal body burden (ng/kg bw) at the end of dosing (Increment over background)	NOEL/LOEL	Reference
Holtzman rats: Decreased ventral prostate weight and decreased anogenital distance in male offspring	Single bolus dose of 12.5 ng/kg bw on gestational day 15	7.5	NOEL	Ohsako et al. 2001
Holtzman rats: Decreased ventral prostate weight and decreased anogenital distance in male offspring	Single bolus dose of 50 ng/kg bw on gestational day 15	30	LOEL	Ohsako et al. 2001
Holtzman rats: Decreased sperm count in male offspring	Single bolus dose of 64 ng/kg bw on gestational day 15	38	LOEL	Mably et al. 1992
Wistar rats: Decreased sperm production and altered sexual behavior in male offspring	Loading dose/ maintenance dose by subcutaneous injections	25	LOEL	Faqi et al. (1998)
F344 rats: Decreased sperm count and accelerated eye opening in male offspring	Single bolus dose of 50 ng/kg bw on gestational day 15	30	LOEL	Gray et al. 1997a
F334 Rats: Immune suppression in male offspring	Single bolus dose of 100 ng/kg bw on gestational day 14	60	LOEL	Gehrs et al. 1997; Gehrs and Smialowicz 1998
F334 Rats: Increased genital malformations in female offspring	Single bolus dose of 200 ng/kg bw on gestational day 15	120	LOEL	Gray et al. 1997b
Rhesus Monkeys: Subtle, non-persistent neurobehavioral (object learning) effect in offspring	0.15 ng/kg bw per day for up to three years	25-37	LOEL	Schantz and Bowman 1989; SCF 2000
Rhesus Monkeys: Endometriosis seen 10 years after secession of dosing	0.15 ng/kg bw per day for up to 3½ years	39	LOEL	Rier et al. 1993; SCF 2000

JECFA (2002) performed benchmark dose modelling of 180 selected experimental datasets to provide a quantitative assessment of the body burdens associated with effects in laboratory animals. The effective dose associated with a 10% increase in the probability of an abnormal response (ED_{10}) was calculated using the USEPA Benchmark Dose Software. The lowest ED_{10} values were derived from the studies performed by Gray et al. (1997a) and Mably et al. (1992) on the rat developmental effects (the studies by Ohsaka et al. (2001) and Faqi et al. (1998) were not included). With the exception of the rat developmental effects data, the ED_{10} body burden values estimated were greater than 200 ng/kg bw.

A conservative (low) estimate of the ED_{01} can be determined by dividing the ED_{10} by 10. Thus, ED_{01}s were estimated to be greater than 20 ng/kg body weight.

Decreased anogenital distance in Holtzman rats (ohsako et al. 2001) and accelerated eye opening and a non-significant decrease (25%) in ejaculated sperm counts in Long Evans rats (Gray *et al*, 1997a) were seen in the male offspring following a maternal bolus dose of 50 ng 2,3,7,8-tetraCDD/kg bw on GD15. In the study by Ohsako *et al.* (2001) pregnant Holtzman rats were given a single oral dose of 0, 12.5, 50, 200 or 800 ng 2,3,7,8-tetraCDD/kg

bw on GD 15, and the male offspring were examined on PND 49 or 120. In this study, there were no changes seen on testicular or epididymal weights nor in daily sperm production or sperm reserve at any of the doses used. However, the weight of the urogenital complex, including the ventral prostate, was significantly reduced at doses of 200 and 800 ng 2,3,7,8-tetraCDD/kg bw in rats sacrificed on PND 120. Moreover, as mentioned the anogenital distance of male rats sacrificed on PND 120 showed a significant decrease in the groups receiving doses of 50 ng 2,3,7,8-tetraCDD/kg or higher. A NOEL of 12.5 ng 2,3,7,8-tetraCDD/kg bw was found in this study. In another study in Holzman rats using a similar protocol, Mably *et al*. (1992) found statistically significant decreases in epididymis and cauda epididymis weights, daily sperm production, and cauda epididymal sperm number in the male offspring after a maternal single gavage dose of 64 ng/kg bw, the lowest dose tested in that study. In these and other single dose gavage studies, an additional number of reproductive and developmental parameters were affected in a dose-related manner in the male offspring at higher dose levels, i.e. from 160 ng 2,3,7,8-tetraCDD/kg bw onwards. At a maternal dose of 200 ng 2,3,7,8-tetraCDD/kg bw or higher Gray *et al*. (1997b) found external malformations of the genitalia in the female offspring. Studies also demonstrate that the effects on the immune system of the male (and female) offspring of pregnant rats exposed to 2,3,7,8-tetraCDD occur only at higher doses than the effects seen on the reproductive organs and their function.

In the study by Faqi *et al*. (1998) Wistar rat dams received an initial loading dose of 25, 60, or 300 ng ^{14}C-2,3,7,8-tetraCDD/kg bw 2 weeks prior to mating, followed by weekly maintenance doses of 5, 12, or 60 ng 2,3,7,8-tetraCDD/kg bw. The size of the maintenance doses was based on a reported elimination half-life of 3 weeks for adult rats. For example, this means that at the low dose the initial loading dose would produce a maternal body burden of 25 ng 2,3,7,8-tetraCDD/kg bw which after one week had declined to 20 ng/kg bw but, following the weekly maintenance dose of 5 ng/kg bw, would again rise to 25 ng/kg bw. After birth, developmental landmarks in the male offspring were monitored. Effects on male reproduction were studied on postnatal days (PND) 70 and 170. The number of sperm per cauda epididymis was reduced in all 2,3,7,8-tetraCDD treated groups at puberty and at adulthood. Daily sperm production was permanently decreased, as was the sperm transit rate in the 2,3,7,8-TCDD exposed male offspring, thus increasing the time required by the sperm to pass through the cauda epididymis. Moreover, the male offspring of the 2,3,7,8-tetraCDD groups showed an increased number of abnormal sperm when investigated at adulthood. There was a lack of a clear dose-response relationship for most of these effects in the treated groups. The fertility of the male offspring was not affected in any of the dosed groups.

In order to estimate the maternal body burden in the pregnant rats of these studies SCF (2000; 2001) and JECFA (2002) used results from studies by Hurst *et al*. (2000a,b). In the first study by Hurst et al. (2000a) ^{3}H-2,3,7,8-tetraCDD concentrations were measured in the tissues of pregnant Long Evans dams at gestational day (GD) 16 following administration by gavage at GD 15 of 0.05, 0.2, 0.8 or 1.0 μg/kg bw. The average maternal body burdens were reported to be 30.6 (60%), 97.4 (48%), 522.8 (65%) or 585.2 (59%) ng 2,3,7,8-tetraCDD/kg bw (percentage of dose), respectively. The corresponding average foetal body burdens at GD16 were 5.3, 13.2, 39.1 and 55.7 ng 2,3,7,8-tetraCDD/kg bw. This study showed absorption of about 60% for 2,3,7,8-tetraCDD in pregnant rats following a single gavage dose.

Both WHO (2002), SCF (2000; 2001) and JECFA (2002) have stressed the importance that the risk assessment of PCDDs, PCDFs, and dioxin-like PCBs be based on the body burden at steady state. As explained by SCF (2000) "The bioavailability of 2,3,7,8-tetraCDD to the foetus at a given maternal body burden may differ between a bolus dose (as in these rat studies) and dietary exposure at steady state. Intuitively, differences in foetal bioavailability would seem likely. Given that placental transfer will be mediated *via* the blood, it is serum rather than tissue levels that will be critical in determining the magnitude of foetal exposure. Following a bolus administration, serum 2,3,7,8-tetraCDD levels would be elevated before redistribution to the tissue compartments. In contrast, low-level chronic exposure will not significantly elevate serum levels. The time of dosing, GD15, marks the onset of the endocrine-sensitive phase of sexual differentiation in rats and therefore represents a critical window for foetal exposure for these reproductive endpoints. […] This would suggest that the critical determinant of these reproductive effects is the foetal concentration on GD15, which, as noted above, is likely to be higher following a single bolus dose on this day than that resulting from lower level chronic exposure. This weakens the relevance to human dietary exposure."

The issue of the difference in magnitude of the foetal body burden following an acute bolus dose compared to that resulting from a low level chronic exposure that leads to a similar maternal body burden was addressed by Hurst *et al*. (2000b) who measured the radioactivity in both the maternal and foetal tissues of pregnant Long Evans dams at GD 9, 16, and 21 following subchronic administration of ^{3}H-2,3,7,8-tetraCDD. Female rats were dosed by gavage with 1, 10, or 30 ng of ^{3}H-2,3,7,8-tetraCDD/kg bw in corn oil, 5 days per week, for 13 weeks. At the end of this period, the rats were mated and dosing was continued every day throughout gestation. The dosage regimen used produced a steady state of 2,3,7,8-tetraCDD in the dams. The average maternal and foetal body burdens at GD 16 are shown in Table 3 and compared with average maternal and foetal body burdens found at GD 16 following the single gavage administration of 2,3,7,8-TCDD on GD 15 in the previous study by Hurst *et al*. (2000a).

As can be seen, acute single gavage doses at GD 15 produced considerably higher foetal concentrations at GD 16 than subchronic administration of low daily doses leading to maternal steady state body burdens of similar magnitude. SCF (2001) and JECFA (2002) analysed the data and performed best-fit analysis of each data set in the range of foetal body burdens from zero to 15.2. It was found that both data sets could be fit to power equations. The equations were used to calculate the corresponding acute and subchronic maternal body burdens for a number of foetal body burdens. From these calculations it was determined that the factor to convert maternal body burden following acute dosing into a corresponding steady state body burden is approximately 2.6 (Table 4). JECFA (2002) also fitted the data to a linear model, which provided somewhat lower estimates of the corresponding subchronic maternal body burdens.

Table 3. Comparison of average maternal and foetal body burdens after single dose and subchronic 2,3,7,8-tetraCDD exposures to pregnant rats

Single dose exposure at GD 15 (1)				Subchronic exposure (2)			
Single dose (3)	Body burden measured at GD 16			Adjusted daily dose (4)	Body burden measured at GD 16		
	Maternal (3)	Foetal (3)	Maternal/ Foetal		Maternal (3)	Foetal (3)	Maternal/ Foetal
50	30	5.3	5.7	0.71	20	1.4	14.3
200	97.4	13.2	7.4	7.1	120	7.5	16.0
800	523	39.1	13.4	21.3	300	15.2	20
1000	585	55.7	10.5				

Data from Hurst *et al*. (2000a)
Data from Hurst *et al*. (2000b)
ng/kg bw
ng/kg bw per day, adjusted to continuous exposure from 5 days/week

Table 4. Corresponding values of foetal, acute maternal and subchronic steady state maternal body burdens of 2,3,7,8-tetraCDD

Foetal body burden (ng/kg bw)	Acute maternal body burden (ng/kg bw)	Subchronic (steady state) maternal body burden (ng/kg bw)	Ratio subchronic maternal/acute maternal body burden
1.2	5.0	12.3	2.5
1.7	7.5	18.6	2.5
1.9	8.5	21.0	2.5
2.1	10	25.0	2.5
3.0	15.5	39.0	2.5
5.3	31	78.6	2.5
6.3	38.5	99.0	2.6
8.0	52	134	2.6
13.2	95.7	251	2.6
15.2	113	299	2.7

Thus, a foetal body burden of 5.3 ng 2,3,7,8-tetraCDD/kg bw, which according to Hurst *et al*. (2000a) was associated with a maternal body burden of 30 ng/kg bw after a single bolus dose at the LOEL of 50 ng/kg bw in the Long Evans rat in the study of Gray *et al*. (1997a) and the study by Ohsako et al. (2001), would correspond to a steady state maternal body burden of approximately 79 ng/kg bw. Similarly, the estimated maternal body burden of 38.5 ng/kg bw after the single gavage LOEL dose of 64 ng/kg bw in the study by Mably *et al*. (1992) corresponds to a foetal body burden of 6.3 ng/kg bw which in turn would require a body burden of approximately 99 ng/kg bw at steady state. The NOEL bolus dose of 12.5 ng 2,3,7,8-tetraCDD/kg bw used in the study by Ohsako et al. (2001) would result in a maternal body burden of 7.5 ng/kg bw. This would translate into a maternal body burden of 19 ng/kg bw at steady state following subchronic daily 2,3,7,8-tetraCDD administration (Table 4).

The intended (pseudo) steady state body burden at the LOEL in the study by Faqi et al. (1998) using subcutaneous administrations was 25 ng 2,3,7,8-tetraCDD/kg bw which, according to Table 4, would correspond to a foetal body burden of 2.1 ng 2,3,7,8-tetraCDD/kg bw. However, following the dosage regimen used, a single maintenance dose of 5 ng/kg bw would have been given at GD 14 when the maternal body burden had declined to 20 ng/kg bw. According to Table 4 a maternal body burden of 20 ng/kg bw in equilibrium corresponds to a subchronic foetal body burden of 1.8 ng/kg bw and the additional acute dose of 5 ng/kg bw during this critical time period in the gestation would produce an extra foetal body burden of 1.2 ng/kg bw, the total foetal body burden thus estimated at 3.0 ng/kg bw. A maternal body burden of 39 ng 2,3,7,8-tetraCDD/kg bw at steady state would be needed to produce this foetal body burden.

Taken together, these studies provide evidence of adverse effects on the reproductive system in the male (and female) offspring of pregnant rats exposed to 2,3,7,8-tetraCDD. The studies demonstrate reduction in daily sperm production, cauda epididymal sperm number and epididymis weight as well as accelerated eye opening, reduction in anogenital distance and feminised sexual behaviour in the male offspring associated with maternal steady state body burdens in the range of 39 – 99 ng 2,3,7,8-tetraCDD/kg bw. In the study of Ohsako *et al*. (2001) a single maternal gavage dose of 12.5 ng 2,3,7,8-tetraCDD/kg bw was identified as a NOEL. This dose produced a decrease in the androgen receptor mRNA level in the ventral prostate at puberty (PND 49), indicative of reduced androgenic responsiveness. However, at this dose level none of the above mentioned adverse effects were seen in the male offspring. This dose corresponds to an estimated maternal steady state body burden of approximately 19 ng 2,3,7,8-tetraCDD/kg bw. As with enzyme induction, altered expression of growth factors and enhanced oxidative stress this effect should be considered an early marker of exposure to 2,3,7,8-tetraCDD or an event induced in animals that may or may not result in adverse effects at higher body burdens.

4.4 Mutagenic and genotoxic effects

2,3,7,8-tetraCDD was mainly negative when tested *in vivo* for chromosomal aberrations, sister chromatid exchanges and increases in the frequency of micronuclei in bone marrow or peripheral lymphocytes in mice, rats or monkeys. A dominant lethal test and tests for DNA adduct formation in rat liver as well as a test for covalent binding of 2,3,7,8-tetraCDD to DNA in mice liver *in vivo* were negative. However, DNA-single strand breaks were observed in rat liver. *In vitro*, tests for reverse mutations in Salmonella

typhimurium (Ames test) and Escherichia coli with and without metabolic activation were predominantly negative. Unscheduled DNA synthesis in human mammary epithelial cells was negative. Test for gene mutations in Saccharomyces cerevisiae, for sister chromatid exchanges in Chinese hamster cells and in human lymphocytes and for micronuclei in human lymphocytes were positive. (ATSDR 1998, IARC 1997).

OctaCDD was negative in Ames test. 1,2,3,6,7,8-hexaCDD and 1,2,3,7,8,9-hexaCDD did not transform C3H 10T1/2 mouse cells. 2,3,4,7,8-pentaCDF increased the frequency of sister chromatid exchanges and micronucleus formation in human lymphocytes *in vitro*. However, 2,3,7,8-tetraCDD did not bind covalently to DNA in mouse liver *in vitro*, and 2,3,4,7,8-pentaCDF as well as 1,2,3,7,8-pentaCDF and 2,3,4,6,7,8-hexaCDF did not bind to DNA in rat liver *in vivo*. Mixtures of PCDDs, PCDFs and PCBs were negative *in vivo* when tested in the mouse spot test but positive in the sister chromatid exchange in human lymphocytes *in vitro*. (IARC 1997).

4.5 Carcinogenic effects

The administration of 2,3,7,8-tetraCDD to rodents significantly increased the incidence of benign as well malignant tumours in various tissues (e.g. liver, thyroid gland, lymphatic system, skin, lungs) in both sexes in several chronic studies. The lowest effective dose causing tumours was 10 ng/kg bw/day for two years at which female rats developed hepatic adenomas. The NOEL was 1 ng/kg/day. In the long-term study in rats in which the incidence of liver tumours was increased, the LOEL (10 ng/kg of body weight per day) corresponded to a steady-state body burden of 294 ng/kg of body weight. In order for humans to attain a similar steady-state body burden, they would have to have a daily intake of 150 pg/kg of body weight (JECFA 2002).

A mixture of 1,2,3,6,7,8-hexaCDD and 1,2,3,7,8,9-hexaCDD fed to mice and rats increased the incidence of hepatocellular adenomas. The number of tumours per animal was small. Administration of several PCDDs, PCDFs and PCBs in combination with known carcinogens enhanced the incidence of tumours and the number of tumours per animals and resulted in the appearance of tumours at earlier times. (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000, JECFA 2002).

A number of hypotheses addressing the mechanism of tumour promotion exist. The PCDDs, PCDFs and PCBs are not acting as initiators of carcinogenesis as evidenced by the mainly negative results in the genotoxicity tests including the lack of covalent binding to DNA. Several indirect mechanism of carcinogenicity are suggested including Ah receptor-mediated alteration in expression of networks of genes involved in cell growth and differentiation, DNA damage mediated by oxidative stress due to induction of cytochrome P450-catalysed metabolic activation pathways, expansion of preneoplastic cell populations via inhibition of apoptosis, positive modulation of intra- or extracellular growth stimuli, or suppression of immune surveillance. Thyroid tumours are probably induced through a mechanism involving induction of hepatic UDP-GT resulting in enhanced elimination of thyroid hormones from the circulation, and consequently elevated levels of circulating thyroid stimulating hormone which results in a chronic proliferative stimulation of thyroid follicular cells. (ATSDR 1998, IARC 1997, SCF 2000, WHO 2000).

5 Regulations, limit values

5.1 Ambient air

-

5.2 Drinking water

-

5.3 Soil

Germany has set a limit value for PCDD/Fs of 5 ng TEQ/kg dry weight. If levels are between 5 and 40 ng TEQ/kg, monitoring should be carried out, and soils with more than 40 ng TEQ/kg are not to be used for agriculture. Furthermore, soils with more than 100 ng TEQ/kg is recommended to be exchanged from playgrounds, soils with more than 1000 ng TEQ/kg is recommended to be exchanged from residential areas, and soils with more than 10,000 ng TEQ/kg is recommended to be exchanged from all locations (MST 1997).

5.4 Occupational Exposure Limits

Denmark: PCB: 0.01 mg/m^3 (HK) (At 2002).

5.5 Classification

PCB is classified for cumulative effects (R33 – danger of cumulative effects), and for environmental effects (N; R50/53 – very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment) (MM 2002).

5.6 EU

Maximum limits for PCDDs and PCDFs have been established by the European Union in food (CR 2000a) and feeding stuffs (CR 2001b). In food, the maximum limits range from 1-3 pg WHO-TEQ/g fat in meat and meat products, as well as milk and egg product. In fish and fish products, the maximum limit was set at 4 pg WHO-TEQ/g fresh weight.

5.7 IARC/WHO

2,3,7,8-tetraCDD is carcinogenic to humans (group 1). Other PCDDs and PCDFs are not classifiable as to their carcinogenicity to humans (group 3) (IARC 1997).

5.8 US EPA

2,3,7,8-tetraCDD is a probable human carcinogen (group B2 alone, group B1 in association with phenoxyherbicides and/or chlorophenols) (ATSDR 1998).

5.9 Risk assessments performed by SCF and JECFA

WHO, the EU Scientific Committee on Food (SCF) and the FAO/WHO Joint Expert Group on Food Additives (JECFA) have recently performed risk assessments of PCDDs, PCDFs and dioxin-like PCBs (WHO 2000, SCF 2000, SCF 2001, JECFA 2002). Although these assessments were performed independently, they used the same approach (body burden approach) and essentially the same pivotal toxicological studies.

These assessments with focus on the SCF (2000, 2001) evaluation are outlined in the following:

5.9.1 Basic considerations

For compounds like PCDDs, PCDFs and PCBs that accumulate in the body fat (and liver), the daily intake rate may not be the appropriate dose metric to use for comparison of different studies and for scaling among different species. Many of the effects following exposure are the same irrespective of the intake being acute or chronic. The toxicity is dependent on the accumulation of small non-toxic doses, resulting in a toxic concentration at the target site. The toxicological responses are directly associated with tissue concentrations and not with the daily dose. The most relevant measure would be the concentration at the target site but it is seldom known. An appropriate surrogate measure might be the total body burden as demonstrated by DeVito et al. (1995). The amount of fat stores in the body, binding to CYP1A2 in the liver, and rate of metabolism and excretion determines the biological elimination half-lives, which varies substantially between experimental animals and humans. Therefore, rodent species require appreciably higher doses (100-200-fold) to reach the same equivalent body burdens as recorded in humans at background exposures. From a pharmacokinetic point of view, body burden estimations are therefore considered a more appropriate dose metric for interspecies comparison than the daily dose.

At low doses it may be anticipated that the body burden of PCDDs, PCDFs and PCBs in a one-compartment model can be adequately described by simple first order kinetics. The relationship between the body burden at steady state and the intake rate is as follows:

Body burden (ng/kg bw) = Intake (ng/kg bw/day) x F x T½ / Ln2
(Equation 1)

Where, F is the fraction absorbed (from dietary exposure estimated to 50% and from exposure via gavage estimated to 60 %) and T½ is the elimination half-life. For humans an elimination half-life of 2740 days (7.5 years) is used. For compounds following first order kinetics it will take 4-5 half-lives to approach steady state.

The above formula (Equation 1) was used by WHO (2000), SCF (2000, 2001) and by JECFA (2002) to calculate the estimated human daily intake

(EHDI) from any given body burden in experimental animals associated with critical effects of 2,3,7,8-tetraCDD.

A number of the effects produced by PCDDs, PCDFs and PCBs in experimental animals and observed in occupationally or accidentally exposed humans are clearly high dose effects and not directly relevant for the evaluation of exposure of the general population at much lower levels. In addition, many of the non-cancer effects observed in mainly adult male workers occupationally exposed to high levels of 2,3,7,8-tetraCDD and higher chlorinated PCDDs were transient effects disappearing after the end of exposure.

A number of biochemical changes such as enzyme induction, enhanced expression of growth factors and enhanced oxidative stress have been noted in experimental animals at body burdens of 3-10 ng/kg bw. While these effects are observed at very low body burdens equivalent to the background exposure, they are not considered appropriate by WHO (2000) and SCF (2000) to use for a derivation of a tolerable daily intake. The biochemical changes, which may or may not result in adverse effects, are considered to be early markers of exposure to PCDDs, PCDFs and PCBs in animals and humans.

Based on limited evidence in humans and sufficient evidence in experimental animals as well as mechanistic considerations the overall evaluation made by IARC (1997) was that there is “limited evidence that 2,3,7,8-tetraCDD is carcinogenic to humans (Group 1)”. However, in human epidemiological studies where an increased incidence of tumours has been observed, the exposure was generally quite high and to mixtures of different PCDDs, phenoxy herbicides and chlorophenols. 2,3,7,8-tetraCDD is not a direct acting genotoxic compound but a tumour-promoting substance. The mechanism behind the tumour promotion probably involves the activation of the Ah receptor. Because of the non-genotoxic character a threshold value for the tumour promoting effect is thought to exist. In a chronic rat study a NOEL for development of hepatic adenomas has been established at 1 ng/kg bw/day, which is equivalent to a body burden of 30 ng/kg bw.

5.9.2 Pivotal studies and critical effects

WHO (2000) and SCF (2000) identified the most sensitive adverse effects of 2,3,7,8-tetraCDD to be developmental and reproductive effects in rats along with endometriosis found in monkeys. These studies are presented in table 2 (chapter 4.3). However, SCF (2001) and JECFA (2002) did not use the monkey studies in their evaluations because new studies cast doubts about the validity of the exposure information.

Single bolus doses of 50-200 ng/kg bw to rats on gestational day 14-15 has caused decreased sperm count, decreased anogenital distance, accelerated eye opening, immune suppression and increased genital malformations in the offspring. A NOEL of 12.5 ng 2,3,7,8-tetraCDD/kg bw (single dose) was identified in one study. Similar effects on male reproductive function were seen in rats following subcutaneous maintenance doses at 25 ng 2,3,7,8-tetraCDD to the dams during pregnancy.

The bioavailability of 2,3,7,8-tetraCDD to the foetus at a given maternal body burden differ between a bolus dose (as in these rat studies) and dietary exposure at steady state. Given that placental transfer will be mediated *via* the blood, it is serum rather than tissue levels that will be critical in

determining the magnitude of foetal exposure. Following a bolus administration, serum 2,3,7,8-tetraCDD levels would be elevated before redistribution to the tissue compartments. In contrast, low-level chronic exposure will not significantly elevate serum levels. Therefore, the foetal concentration on the sensitive GD15 is higher following a single bolus dose on this day than that resulting from lower level chronic exposure.

SCF (2001) provided a means to overcome this problem and convert the body burdens resulting from these acute exposures into corresponding estimated maternal steady state body burdens from chronic exposures (see chapter 4.3). From these calculations it was determined that the factor to convert maternal body burden following acute dosing into a corresponding steady state body burden is approximately 2.5 (Table 4, chapter 4.3).

The estimated maternal steady state body burdens at NOAEL and LOAEL for the pivotal studies are shown in Table 5. In Table 5 is also given the estimated associated human daily intakes (EHDI), calculated using the formula given above (Equation 1).

In summary, the pivotal studies provide a NOAEL and LOAELs for the most sensitive adverse effects of 2,3,7,8-tetraCDD exposures in experimental animals, i.e. developmental effects in rat male offspring. The sensitive adverse responses (LOAELs) were associated with steady state body burdens between 39 and 99 ng 2,3,7,8-tetraCDD/kg bw with associated estimated human daily intakes (EHDI) in the range of 19.5 - 49.5 pg 2,3,7,8-tetraCDD/kg bw (see Table 3). For the NOAEL as observed in the study of Ohsako *et al*. (2001) a maternal steady state body burden of 19 ng/kg bw and an associated EHDI of 9.5 pg 2,3,7,8-tetraCDD/kg bw was calculated (Table 5) (SCF 2001, JECFA 2002).

TABLE 5. Estimated animal steady state body burdens of 2,3,7,8-tetraCDD and associated estimated human daily intakes (EHDI) at NOAELs and LOAELs in the pivotal studies.

Study	Endpoint	NOAEL	LOAEL	Estimated maternal steady state body burden (ng/kg bw) [1)]	Associated EHDI (pg/kg bw)
Mably *et al.*, 1992	Holzman rats: Decreased sperm count in male offspring		64 ng/kg bw single bolus dose by gavage	99 [2)]	49.5
Gray *et al.*, 1997a	Long Evans rats: Accelerated eye opening and decreased sperm count in male offspring		50 ng/kg bw single bolus dose by gavage	79 [2)]	39.5
Faqi *et al*., 1998	Wistar rats: Decreased sperm production and altered sexual behavior in male offspring		Maintenance of 25 ng/kg bw by subcutaneous injections	39 [2)]	19.5
Ohsako *et al.,* 2001	Holzman rats: Decreased anogenital distance in male offspring	12.5 ng/kg bw single bolus dose by gavage		19 [3)]	9.5
			50 ng/kg bw single bolus dose by gavage	79 [3)]	39.5

[1)] Increment over background. Background body burden in rats is about 4 ng TEQ/kg bw (WHO, 2000; JECFA 2002).
[2)] Composite value resulting from of pseudo steady state body burden and acute body burden on GD 15.
[3)] Maternal body burden at gestation day 16.

5.9.3 Derivation by SCF of a TDI for 2,3,7,8-tetraCDD from the sensitive NOAEL (SCF 2001)

$$TDI = \frac{EHDI}{SF_I \times SF_{II} \times SF_{III}} = \frac{9.5}{1 \times 3.2 \times 1} = 3 \text{ pg /kg bw/day}$$

The safety factor SF_I was set to 1. Studies of Ah receptor binding affinity and adverse responses directly dependent on Ah receptor activation suggest that humans are less sensitive to 2,3,7,8-tetraCDD than responsive rodent strains. However, studies of some biochemical or cellular effects, such as CYP1A1 and CYP1A2 induction, suggest a comparable sensitivity. Therefore, for some endpoints it cannot be excluded that the most sensitive humans might be as sensitive to the adverse effects of 2,3,7,8-tetraCDD as experimental animals. Therefore, no safety factor in either direction needed to be applied for differences in toxicodynamics among rats and humans and for differences within the human population. In addition, the use of an uncertainty factor to account for differences between rats and humans in toxicokinetics was not required since the default toxicokinetic factor was replaced by actual data in calculating the body burdens used to scale doses across species.

The SF_{II} was set to 3.2 to account for differences in sensitivity among individuals of the human population because of variability in the toxicokinetics. There are only limited data available on the toxicokinetics of 2,3,7,8-tetraCDD in humans, therefore the default factor of 3.2 as suggested by WHO (1994) was considered appropriate.

No additional SF_{III} was needed because the NOAEL used represents the most sensitive effect seen in an extensive toxicological database on 2,3,7,8-tetraCDD (SCF 2001).

5.9.4 Derivation by SCF of a TDI for 2,3,7,8-tetraCDD from the most sensitive LOAEL (SCF 2001)

$$TDI = \frac{EHDI}{SF_I \times SF_{II} \times SF_{III}} = \frac{19.5}{1 \times 3.2 \times 3} = 2 \text{ pg /kg bw/day}$$

In using the most sensitive LOAEL of 19.5 pg 2,3,7,8-tetraCDD instead of the NOAEL an additional uncertainty factor needed to be applied. As the LOAELs reported for the sensitive endpoints were considered to be close to the NOAEL and to represents marginal effects a factor of 3 was considered appropriate to account for the use of a LOAEL instead of a NOAEL. In this case, an overall uncertainty factor of 9.6 was applied (SCF 2001).

5.9.5 Derivation of tolerable intakes for PCDDs, PCDFs and dioxin-like PCBs (SCF 2001, JECFA 2002)

SCF (2001) recognised that the Wistar rats as used in the study by Faqi *et al*. (1998) might be the most sensitive rat strain. Therefore 2 pg/kg bw per day was considered as a tolerable intake for 2,3,7,8-tetraCDD. The TDI for 2,3,7,8-tetraCDD was extended to cover all dioxins and dioxin-like compounds, expressed as WHO-TEQ, because the differences in half-lives between the dioxins and dioxin-like compounds are small and partly accounted for in the establishment of the TEF values. Thus, a TDI of 2 pg WHO-TEQ/kg bw per day was established by SCF (2001) for the sum of PCDDs, PCDFs and dioxin-like PCBs.

In the evaluation performed by JECFA (2002) a weighted average tolerable intake (corresponding to a TDI of 2.3 pg 2,3,7,8-tetraCDD/kg bw) was derived from the most sensitive animal studies (JECFA 2002).

It should be noted that these tolerable intakes were established on the basis of body burdens in the rat that were increments to background levels. The background WHO-TEQ body burden in the rat is in the range of 4-12 ng/kg bw, depending on the feed offered to the animals (JECFA 2002). As a daily intake at the TDI level (2 pg WHO-TEQ/kg bw/day) in humans will result in a steady state body burden of 4 ng/kg bw (Equation 1, paragraph 5.9.1) it can be argued that an additional safety factor of at least 2 is hidden in the TDI.

Both SCF (2001) and JECFA (2002) considered that for compounds like 2,3,7,8-tetraCDD and related substances that have very long half-lives in the human body the tolerable intake should be expressed on a weekly (SCF) or monthly (JECFA) rather than on a daily basis. Therefore SCF established a tolerable weekly intake (TWI) of 14 pg 2,3,7,8-TCDD/kg bw whereas JECFA established a provisional monthly tolerable intake (PTMI) of 70 pg 2,3,7,8-tetraCDD/kg bw. In both evaluations the tolerable intakes for 2,3,7,8-tetraCDD were extended to cover all dioxins and dioxin-like compounds, expressed as WHO-TEQ.

6 Summary

6.1 Description

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofuranes (PCDFs) and dioxin-like biphenyls (PCBs) are chemical compounds with similar structures. They elicit toxic effects by a common mechanism. The different structurally related molecules are referred to as congeners of one another.

PCDDs, PCDFs and PCBs are colourless or yellow solids or crystals. In general all the PCDDs, PCDFs and PCBs are lipophilic. They have very low water solubility and vapour pressure.

Since the 1930es and until the middle of the 1970es, PCBs have been used extensively in various products for example in electric equipment and in hydraulic systems. Today the PCBs have been replaced in a lot of products. PCDDs and PCDFs are only used in chemical and toxicological research. However, they are by-products of combustion processes, such as municipal waste incineration, and of various industrial processes in the chemical industry for instance during the production of PCBs.

6.2 Environment

PCDDs, PCDFs and PCBs do not occur naturally in the environment but because of their high persistence they are present all over the world as complex mixtures of various congeners. Some photolytic degradation of the PCDDs, PCDFs and PCBs may occur in the surface layer of soil or in the air. However, most of the PCDDs, PCDFs and PCBs are deposited in soil or water. For instance the half-life in soil of the most toxic congener, 2,3,7,8-tetraCDD, has been estimated to be more than 10 years.

Since dioxins and dioxin-like PCBs are very lipophilic and extremely resistant towards chemical and biological degradation processes, they persist in the environment and accumulate in food chains.

6.3 Human exposure

Over 90 % of the general human exposure to dioxins and dioxin-like PCBs is estimated to occur through the diet. Fatty fish, milk and dairy products and meat are the main foods containing the congeners. The average daily intake of PCDDs, PCDFs and dioxin-like PCBs in various North European countries has been estimated to be in the range of 1.2-3.3 pg WHO TEQ/kg bw per day. Due to the accumulation of PCDDs, PCDFs and PCBs in human fat, human milk can contain high concentrations of these compounds. This means that the suckling baby may ingest as much as 180 pg I-TEQ/kg bw/day, assuming an intake of 6 g fat/kg bw/day from mothers milk.

Within the past few years, a substantial reduction in the intake of PCDDs, PCDFs and PCBs has occurred reflecting the reduction of emissions in the late 1980es.

6.4 Toxicokinetics

Molecular size and solubility of a congener are the rate limiting factors for the absorption from the gastrointestinal tract. Congeners having 4, 5, or 6 chlorine atoms are well absorbed (50-90 %) while hepta-and octa chlorinated congeners are absorbed to a lesser extent.

Many PCDDs, PCDFs and PCBs are very resistant to metabolism. PCDFs are more susceptible to biochemical degradation than PCDDs because of the stress on the furan ring. Elimination takes place through bile and faeces in the form of hydroxylated or conjugated metabolites.

However, due to the high lipophilicity and resistance to biotransformation, most of the congeners accumulate in the body, mainly in fat tissue and liver. In humans, the half-life of 2,3,7,8-tetraCDD has been reported to range from 5.5 to 11 years whereas in rats, the half-life ranged from 17 to 31 days and in monkeys it is about 400 days. Congeners can pass the placenta of pregnant animals and humans.

The biochemical and toxic effects of the PCDDs, PCDFs and PCBs are mediated through the binding of the congeners to the intracellular aryl hydrocarbon (Ah) receptor. When xenobiotics bind to and activate the receptor, the result is various biochemical responses including modulation of growth factors, thyroid hormones, protein phosphorylation, glucose metabolism, estrogenic responses, lipid peroxidation, cell cycle regulation and apoptosis as well as induction of drug-metabolising enzymes.

6.5 Human toxicity

Many epidemiological data exist for exposure to PCDDs, PCDFs and PCBs in chemicals workers, Viet Nam veterans and the general population in connection with industrial accidents or contaminations with mixtures of congeners.

Chloracne is the most widely recognised dermal effect of exposure to PCDDs, PCDFs and PCBs in humans. It occurs shortly after acute or chronic exposure to a variety of chlorinated aromatic compounds by skin contact, ingestion or inhalation. Other effects observed in some but not all of the studies include an increased incidence of cardiovascular disease, an increased risk for diabetes, an increased mortality from chronic liver disease and cirrhosis, immunological alterations, ophthalmological changes, chronic bronchitis, respiratory infections and alterations in the level of thyroid hormones. Transient nausea, vomiting and abdominal pain, neurological symptoms as well as elevations in liver enzyme have also been observed.

6.5.1 Toxicity to reproduction

Most studies on reproductive effects of PCDDs in humans concerned paternal exposure of workers or Viet Nam veterans. Some studies have shown alterations in sex hormone levels and sperm characteristics after PCDD exposure. Discordant results exist for an increase in the risk of spontaneous abortions among the wives of the exposed men. In most of these studies, an elevation in birth defects was not detected. However, in children born of parents exposed to contaminated rice oil in Japan and Taiwan, an increased incidence of e.g. developmental delays and mild persistent behaviour disorders was observed. Neurodevelopmental delays and

neurobehavioral effects also occurred in the most highly exposed infants in three cohorts with measured background exposure to PCDDs, PCDFs and/or PCBs. An alteration of the sex ratio (more females than males) was observed for children born to parents highly exposed to 2,3,7,8-tetraCDD after an industrial accident in Seveso. The effect was reported to be paternal.

6.5.2 Carcinogenicity

Increased risks for all cancers combined were seen in the occupational cohort studies. The magnitude of the increase was generally low. However, it was higher in sub-cohorts considered to have the heaviest 2,3,7,8-tetraCDD exposures. In most of the cancer studies excess risks were observed for soft tissue sarcoma, lung cancer, non-Hodgkin lymphoma and digestive tract cancers. Statistically significant excess risks were observed in individual cohorts for a variety of other cancers.

JECFA (2002) explored the calculation of a "benchmark dose" (e.g., the ED_{01}, the dose estimated to result in a 1% increase in cancer mortality), on the basis of a meta-analysis of data from three industrial cohorts with well-documented exposure to TCDD. A statistically significant linear trend in risk with exposure was observed, which persisted even after exclusion of groups with the highest exposure. The ED_{01} ranged quite widely and strongly depended on the assumptions made. JECFA (2002) estimated an ED_{01} at 41 pg/kg/day (90% CI: 22, 131). This value is best interpreted as an exposure above the current background TCDD-TEQ level that predicts to increase the risk of cancer mortality by 1% above the current level (which includes any contribution by background levels of TCDD-TEQ to background cancer mortality). JECFA also cited assessments performed by the U.S. Environmental Protection Agency (USEPA 2000) and by Steenland *et al.* (2001). USEPA estimated an ED_{01} corresponding to a daily intake of 5.9 pg/kg bw/day (95% lower bound = 3.7 pg/kg bw/day). Steenland *et al.* (2001) predicted an ED_{01} corresponding to a daily intake of 14 pg/kg bw/day.

6.6 Animal toxicity

Lethality is the effect that varies most among species, i.e. the LD50 varies from 1 μg/kg bw in the guinea pig to >1000 μg/kg bw in the hamster. In all mammalians species tested so far, lethal doses of PCDDs and PCDFs results in delayed death preceded by an excessive body weight loss. Other signs of intoxication include thymic atrophy, hypertrophy/ hyperplasia of hepatic, gastrointestinal, urogenital and cutaneous epithelia, atrophy of the gonads, subcutaneous oedema, and systemic haemorrhage.

Relatively high doses of PCDDs, PCDFs and PCBs have resulted in chloracne, altered cardiac function and morphology, disturbances of the sex steroids, corticosteroids and thyroid hormones, fatty infiltration and necrosis of the liver, and/or neurological signs in experimental animals.

A number of biochemical changes such as enzyme induction, enhanced expression of growth factors and enhanced oxidative stress have been noted in experimental animals at body burdens of 3-10 ng/kg bw. Inconsistent immunological effects have also been noted at low doses.

6.6.1 Toxicity to reproduction

PCDDs, PCDFs and PCBs are developmental and reproductive toxicants in experimental animals. Most perturbations of the reproductive system in adult animals require overtly toxic doses. In contrast, effects on the developing organism occur at doses more than 100 times lower than the doses producing toxic effects in the mother. Sensitive targets include the developing reproductive, nervous and immune systems. Perturbations of multiple hormonal systems and their metabolism may play a role in these events.

The most sensitive adverse effects of 2,3,7,8-tetraCDD in animals are developmental and reproductive effects. Single bolus doses of 50-200 ng/kg bw to rats on gestational day 14-15 has caused decreased sperm count, accelerated eye opening, immune suppression and increased genital malformations in the offspring. A single bolus dose of 12.5 ng/kg bw was a NOEL.

JECFA (2002) performed benchmark dose modelling of 180 selected experimental datasets to provide a quantitative assessment of the body burdens associated with effects in laboratory animals. The effective dose associated with a 10% increase in the probability of an abnormal response (ED_{10}) was calculated using the USEPA Benchmark Dose Software. The lowest ED_{10} values were derived from the studies on the developmental effects in the rat. With the exception of the rat developmental effects data, the ED_{10} body burden values estimated were greater than 200 ng/kg bw.

Neurobehavioral effects were reported in the offspring of female Rhesus monkeys fed 0.15 ng/kg bw per day of 2,3,7,8-tetraCDD for up to 3½ years. At follow-up after 10 years the Rhesus Monkey dams that had been exposed to 2,3,7,8-tetraCDD showed an increased incidence of endometriosis compared to control monkeys. However, recent studies have raised a number of questions about the actual exposures that these monkeys have experienced, and the studies were not used by SCF (2001) in its up-dated risk assessment or by JECFA (2002).

6.6.2 Mutagenic and genotoxic effects

PCDDs, PCDFs and PCBs have been tested in various tests *in vitro* and *in vivo* in research animals and humans for mutagenic and genotoxic effects. Most of the tests were negative. In addition, the lack of covalent binding of PCDDs and PCDFs to DNA has been demonstrated.

6.6.3 Carcinogenicity

The administration of 2,3,7,8-tetraCDD (as well as a few other PCDDs, PCDFs and PCBs) to rodents significantly increased the incidence of benign as well malignant tumours in various tissues in several chronic studies. The lowest dose causing tumours was an intake of 10 ng/kg bw/day for two years at which female rats developed hepatic adenomas. The NOEL was 1 ng/kg/day which is equivalent to a body burden of 30 ng/kg bw.

The PCDDs, PCDFs and PCBs are not acting as initiators of carcinogenesis. A number of hypotheses exist that are addressing the mechanism of tumour promotion via indirect mechanisms involving the activation of the Ah-receptor.

6.7 Recent evaluations by SCF and JECFA

The EU Scientific Committee on Food (SCF) and the FAO/WHO Joint Expert Group on Food Additives have recently performed risk assessments of PCDDs, PCDFs and dioxin-like PCBs (SCF 2000, SCF 2001, JECFA 2002). Although these assessments were performed independently, they used the same approach (body burden approach) and essentially the same pivotal toxicological studies. The SCF arrived at a tolerable weekly intake of 14 pg WHO-TEQ/kg bw (corresponding to a TDI of 2 pg WHO-TEQ/kg bw/day) and the JECFA established a tolerable monthly intake of 70 pg WHO-TEQ/kg bw (corresponding to a TDI of 2.3 pg WHO-TEQ/kg bw/day).

7 Quality criterion in soil

No health based quality criterion is set for PCDDs, PCDFs and dioxin-like PCBs (expressed as WHO-TEQ) in soil for the following reasons:

Quality criteria for chemicals in soil are primarily aimed at protecting children who may come into dermal contact with the soil or children who may ingest it. However, these compounds would normally be firmly bound to particles in soil and dermal absorption is considered to be low. Thus, only about 4% of radio labelled Aroclor 1260 (a complex PCB mixture) mixed into a typical US soil was absorbed following dermal application to Rhesus monkeys (Mayes et al. 2002). Following oral ingestion the absorption will also be low due to the binding of the compounds to soil components, however, even if absorption is considered as high as from fatty foods (considered to be 50%) no acute or subchronic effects are anticipated to occur in children from the levels currently occurring in soil (0.25 - 3 ng I-TEQ/kg dry weight which approximately equals 0.25 – 3 ng WHO-TEQ/kg dry weight). The critical effects (altered hall-marks of sexual development and decreased sperm production in male offspring) seen in experimental animals and used in the TDI-derivation (see Table 5) are related to the body burden in the adult, pregnant female, and the implication of the effects being related to their male offspring. The derivation of the TDI for PCDDs, PCDFs and dioxin-like PCBs was therefore based on the body burden approach, where the sensitive LOEL or NOEL animal body burdens were converted into estimated human daily intakes that would lead to the same body burdens at steady state in humans. The PCDDs, PCDFs and dioxin-like PCBs have very long half-lives in the human body (in the order of 7.5 years for 2,3,7,8-tetraPCD), and it will take 4-5 half lives to attain steady state. Therefore, elevated exposures within a shorter time period, i.e. in toddlers, will not have any significant impact on the overall steady state body burden obtained after 30-40 years.
The following scenarios may serve to illustrate this:

The general population is mainly exposed to PCDDs, PCDFs and PCBs from food and an intake at the TDI level of 2 pg WHO-TEQ/kg bw/day, derived by the SCF (2001) from food will result in a steady state body burden of 4 ng WHO-TEQ/kg bw after 30-40 years (Equation 1, chapter 5.9.1). It is this *tolerable body burden* that should not be significantly exceeded in the pregnant woman in order to protect against the detrimental effects of dioxins.

In Denmark, Vikelsøe (2002) found between 0.25 – 3 ng I-TEQ PCDDs and PCDFs per kg dry weight in soils (approximately equal to 0.25 – 3 ng WHO-TEQ/kg dry weight) from urban areas, considered to be contaminated, with only three samples being above 1 ng I-TEQ per kg dry weight. The mean concentration of samples from rural areas, considered to reflect background contamination, was 0.67 ng I-TEQ per kg dry weight.

Scenario 1:
For ease of calculation, it is assumed that the background contamination of soil is 0.5 ng WHO-TEQ/kg soil. If it is anticipated that a child having a body weight of 10 kg will ingest 0.2 g soil per day, a background level of 0.5 ng/kg soil will provide an additional intake of 0.01 pg WHO-TEQ/kg bw/day. If this additional per kg bw/day intake from soil continues for 30-40

years (which is highly unlikely), this would result in an additional body burden of 0.02 ng WHO-TEQ/kg bw (Equation 1, chapter 5.9.1) on top of the body burden of 4 ng/kg bw/day obtained from food intake, resulting in a total body burden of 4.02 ng WHO-TEQ/kg bw.

Scenario 2:
It is assumed that a contaminated soil contains 3 ng WHO-TEQ/kg soil (considered to be a high contamination level in Denmark). A daily intake of 0.2 g soil results in a daily intake of 0.6 pg WHO-TEQ per child equivalent to 0.06 pg WHO-TEQ/kg bw/day for a child weighing 10 kg. If this additional intake (on a per kg bw basis) continued until steady state (30-40 years) an additional body burden of 0.12 ng WHO-TEQ/kg bw will be obtained (Equation 1, chapter 5.9.1) resulting in a total body burden of 4.12 ng WHO-TEQ/kg bw.

Scenario 3:
It is assumed that a contaminated soil contains 3 ng WHO-TEQ/kg soil (considered to be a high contamination level in Denmark). A daily intake of 0.2 g soil results in a daily intake of 0.6 pg WHO-TEQ per child equivalent to 0.06 pg/kg bw/day for a child weighing 10 kg. If this additional intake by the child (on a per kg bw basis) continued for only 2 years (730 days) the total additional intake would be approximately 50 pg WHO-TEQ/kg bw (0.05 ng WHO-TEQ/kg bw) over this time period. If no excretion of absorbed WHO-TEQ took place the total body burden at steady state after 30 years would thus be 4.05 ng WHO-TEQ/kg bw. However, after 30 years (4 half-lives of 7.5 years each) the additional 0.05 ng WHO-TEQ/kg bw would have been reduced to approximately 0.003 ng WHO-TEQ/kg bw, resulting in a total body burden of 4.003 ng WHO-TEQ/kg bw.

The two “worst case” scenarios 1 and 2 and the, still conservative, but maybe more realistic scenario 3 clearly shows that the contribution from soil to intake of PCDDs, PCDFs and dioxin-like PCBs will have a negligible impact on the critical body burden of PCDDs, PCDFs and dioxin-like PCBs. If a quality criterion for PCDDs, PCDFs and dioxin-like PCB in soil was to be enforced in order to prohibit that no more than an additional 1% of the body burden of 4 ng WHO-TEQ/kg (total body burden of 4.04 ng WHO-TEQ/kg bw) should be obtained from ingestion of soil, a value of 40 ng WHO-TEQ/kg soil would be required based on a calculation using the premises outlined in scenario 3. Such a high contamination levels is considered unlikely to occur to an appreciable extent for soils in Denmark. Therefore there is no need to set a health based soil quality criterion based on children’s direct exposure to soil for PCDDs, PCDFs and dioxin-like PCBs.

8 References

At (2002). Grænseværdier for stoffer og materialer. Arbejdstilsynet At-vejledning C.0.1 Oktober 2002.

ATSDR (1998). Toxicological Profile for Chlorinated Dibenxo-p-dioxins (Update). U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR (1997). Toxicological Profile for Polychlorinated Biphenyls (Update). U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

CR (2001a). Council Regulation 2001/2375/EC of 29 November 2001 amending Commission Regulation N^0 466/2001 setting maximum limits for certain contaminants in food.

CR (2001b). Council Regulation 2001/102/EC of 27 November 2001 amending Council Regulation 1999/29/EC setting maximum limits for certain contaminants in feed.

DeVito MJ, Birnbaum LS, Farland WH and Gasiewicz TA (1995). Comparisons of estimated human body burdens of dioxin-like chemicals and TCDD body burdens in experimentally exposed animals. Environ Health Perspect 101, 820-831.

Faqi AS, Dalsenter PR, Merker H. and Chahoud I (1998). Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicol Appl Pharmacol 150(2), 383-92.

Gehrs BC, Riddle MM, Williams WC and Smialowicz RJ (1997). Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. II. Effects on the pup and the adult. Toxicology 122, 229-240.

Gehrs BC and Smialowicz RJ (1998). Persistent suppression of delayed-type hypersensitivity (DTH) in rats perinatally exposed to TCDD. Toxicologist 42, 1501.

Gray LE, Ostby JS and Kelce WR (1997a). A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans hooded rat offspring. Toxicol Appl Pharmacol 146, 11-20.

Gray LE, Wolf C, Mann P and Ostby JS (1997b). *In utero* exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters reproductive development of female Long Evans hooded rat offspring. Toxicol Appl Pharmacol 146, 237-244.

Hurst CH, De Vito MJ, Setzer RW and Birnbaum L (2000a). Acute administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in pregnant

Long Evans rats: Association of measured tissue concentrations with developmental effects. Toxicol Sci 53, 411-420.

Hurst CH, DeVito MJ, and Birnbaum LS (2000b). Tissue disposition of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing Long-Evans rats following subchronic exposure. Toxicol Sci 57, 275-283.

IARC (1997). Polychlorinated Dibenzo-para-dioxins and polychlorinated dibenzofurans. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, volume 69, World Health Organisation, International Agency for Research on Cancer, Lyon.

IPCS (1993). Polychlorinated Biphenyl and Terphenyls (Second edition). Environmental Health Criteria 140. World Health Organisation, International Programme on Chemical Safety, Geneva.

JECFA (2002). Polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls. WHO Food Additives Series. Safety evaluation of certain food additives and contaminants. Prepared by the Fifty Seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). International Programme on Chemical Safety (IPCS), World Health Organization, Geneva, pp 451-664.

Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A and Peterson RE (1992). *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. III Effects on spermatogenesis and reproductive capability. Fund Appl Toxicol **21**, 433-441.

MM (2002). The Statutory Order from the Ministry of the Environment no. 439 of June 3, 2002, on the List of Chemical Substances.

MST (1997). Dioxins. Working report nr. 50. Ministry of Environment and Energy, Danish Environmental Protection Agency.

MST (2000). Substance flow analysis for dioxins in Denmark. Environmental project no. 570. Erik Hansen (COWI). Ministry of Environment and Energy, Danish Environmental Protection Agency. Http://www.mst.dk/udgiv/Publications/2000/87-7944-295-1/pdf/87-7944-297-8.PDF

Mayes BA, Brown GL, Mondello FJ, Holtzclaw KW, Hamilton SB and Ramsey AA (2002). Dermal absorption in Rhesus monkeys of polychlorinated biphenyls from soil contaminated with Aroclor 1260. Regul Toxicol Pharmacol 35, 289-295.

Neubert D (1997/98). Reflections on the assessment of the toxicity of “dioxins” for humans, using data from experimental and epidemiological studies. Teratogenesis, Carcinog Mutagen 17, 157-215.

Ohsako S, Miyabara Y, Nishimura N, Kurosawa S, Sakaue M, Ishimura R, Sato M, Takeda K, Aoki Y, Sone H, Tohyama C and Yonemoto J (2001). Maternal Exposure to a Low Dose of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Suppressed the Development of Reproductive Organs of Male Rats: Dose-Dependent Increase of mRNA Levels of 5 ?-Reductase Type 2 in Contrast to Decrease of Androgen Receptor in the Pubertal Ventral Prostate. Toxicol Sci 60, 132-143.

Rier SH, Martin DC, Bowman RE, Dmowski WP and Becker JL (1993). Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundam Appl Toxicol 21, 433-441.

Rier SE, Turner WE, Martin DC, Morris R, Lucier GW and Clark GC (2001). Serum levels of TCDD and dioxin-like chemicals in rhesus monkeys chronically exposed to dioxin: corrrelation of increased serum PCB levels with endometriosis. Toxicol Sci 59, 147-159.

SCF (2000). Opinion of the Scientific Committee on Food (SCF) on the risk assessment of dioxins and dioxin-like PCBs in food. Adopted on 22nd November 2000. Http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html

SCF (2001). Opinion of the Scientific Committee on Food (SCF) on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since the adoption of the SCF opinion of 22nd November 2000. Adopted on 30 May 2001. Http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html

Schantz SL and Bowman RE (1989). Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotoxicol Teratol 11, 13-19.

SCOOP (2000). Reports on tasks for scientific cooperation. Assessment of dietary intake of dioxins and related PCBs by the populations of EU member states. Report of experts participating in task 3.2.5. 7 June 2000. Http://europa.eu.int/comm/dgs/health_consumer/library/pub/pub08_en.pdf

Sundhedsstyrelsen (1999). Indhold af dioxiner, PCB, visse chlorholdige pesticider, kviksølv og selen i modermælk hos danske kvinder 1993-94. Sundhedsstyrelsen, Fødevaredirektoratet.

Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenck D, Tillitt D, Tysklind M, Younes M, Wærn F and Zacharewski T (1998). Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and for Wildlife. Environ. Health Perspec. 106, 775-792.

WHO (2000). Assessment of the health risk of dioxins: re-evaluation of the Tolerable Daily Intake (TDI). WHO Consultation May 25-29 1998, Geneva, Switzerland. WHO European Centre for Environment and Health and International Programme on chemical safety. World Health Organization, Geneva. Food Additives Contaminants **17**.

USEPA (2000). Health Assessment of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds. Draft 9/18/2000. http://www.epa.gov/ncea/pdfs/dioxin/dioxreass.htm

Vikelsøe, J (2002). Dioxins in Danish soil. National Environmental Research Institute, P.O.Box 358, 4000 Roskilde, Denmark. Http://www.mst.dk/news/09100000.htm (26-06-2002).

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs)
The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to poly chlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls. This resulted in 2004 in the present report which includes estimation of a quality criterion for the mentioned compounds in soil.

Danish Ministry of the Environment
Environmental Protection Agency

Strandgade 29
1401 Copenhagen K, Denmark
Tel.: (+45) 72 54 40 00

www.mst.dk