Review

Risk assessments of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in food

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The polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and dioxin-like polychlorinated biphenyls (dioxin-like PCB) are ubiquitous in food of animal origin and accumulate in fatty tissues of animals and humans. The most toxic congener is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and reproductive and developmental toxicity. Toxic equivalency factors have been established for the other PCDD, PCDF and dioxin-like PCB relative to TCDD, and the combined toxicity of a sample can be expressed as toxic equivalent (WHO-TEQ). The EC Scientific Committee for Food evaluated these compounds in 2001. The assessment used the most sensitive adverse toxicological end-points of TCDD in experimental animals. These were developmental and reproductive effects in the male offspring of rats administered TCDD during pregnancy. Because of the large difference between rats and humans in the biological half-life of TCDD, the assessment used a body burden approach to compare across species and derived a tolerable weekly intake of 14 pg TCDD/kg of body weight (bw), which was extended to include all the 2,3,7,8-substituted PCDD and PCDF, and the dioxin-like PCB, and expressed as a group tolerable weekly intake of 14 pg WHO-TEQ/kg bw. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) performed a similar assessment whereas the US Environmental Protection Agency (US EPA) has paid more attention to human data on carcinogenicity.

Keywords: Dioxins / Food / Furans / Polychlorinated biphenyls / Risk assessment

Received: November 21, 2005; revised: March 17, 2006; accepted: March 20, 2006

1 Introduction

The toxic potencies of the individual polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners ("dioxins") differ considerably. The congeners that are of toxicological importance are substituted in each of the 2-, 3-, 7-, and 8-positions. Thus, among the 210 theoretically possible congeners, only 17 are of toxicological concern. They have a toxicological profile similar to that of the most toxic congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and reproductive and developmental toxicity. The toxicity is mediated via the arylhydrocarbon receptor (AhR) present in most tissues of animals and humans. Among the 209 theoretically possible polychlorinated biphenyl (PCB) congeners 12 non-*ortho* and mono-*ortho* substituted PCB show toxicological properties similar to 2,3,7,8-TCDD and are therefore termed dioxin-like PCB.

Dioxins and PCB are lipophilic compounds. They are very resistant towards chemical and biological degradation processes and therefore persist in the environment, accumulate in food chains, and consequently end up in fatty tissues of animals and humans.

In the following, a summary is given of the most recent European risk assessments of PCDD, PCDF, and dioxin-like

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Abbreviations: **AhR**, arylhydrocarbon receptor; **bw**, body weight; **EHDI,** estimated human daily intake; **GD**, gestational day; **JECFA**, FAO/WHO Joint Expert Committee on Food Additives; **LOAEL**, lowest-observed-adverse-effect-level; **NOAEL**, no-observed-adverse-effect-level; **PCB**, polychlorinated biphenyls; **PCDD**, polychlorinated dibenzo-*p*-dioxins; **PCDF**, polychlorinated dibenzofurans; **SCF**, EC Scientific Committee for Food; **TCDD**, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; **TEF**, toxic equivalency factors; **TEQ**, toxic equivalents; **US EPA**, US Environmental Protection Agency

Table 1. Toxic equivalency factors (TEF) for PCDD, PCDF, and dioxin-like PCB established by WHO [11][a)]

PCDD	TEF	PCB (IUPAC number) Non-*ortho PCB*	TEF
2,3,7,8-TCDD	1	3,3′,4,4′-TCB (77)	0.0001
1,2,3,7,8–PnCDD	1	3,4,4′,5-TCB (81)	0.0001
1,2,3,4,7,8–HxCDD	0.1	3,3′,4,4′,5–PnCB (126)	0.1
1,2,3,6,7,8–HxCDD	0.1	3,3′,4,4′,5,5′-HxCB (169)	0.01
1,2,3,7,8,9–HxCDD	0.1		
1,2,3,4,6,7,8–HpCDD	0.01		
OCDD	0.0001		
PCDF	TEF	Mono-*ortho PCB*	TEF
2,3,7,8-TCDF	0.1	2,3,3′,4,4′-PnCB (105)	0.0001
1,2,3,7,8-PnCDF	0.05	2,3,4,4′,5-PnCB (114)	0.0005
2,3,4,7,8-PnCDF	0.5	2,3′,4,4′,5-PnCB (118)	0.0001
1,2,3,4,7,8-HxCDF	0.1	2,3,4,4′,5-PnCB (123)	0.0001
1,2,3,6,7,8-HxCDF	0.1	2,3,3′,4,4′,5-HxCB (156)	0.0005
1,2,3,7,8,9-HxCDF	0.1	2,3,3′,4,4′,5′-HxCB (157)	0.0005
2,3,4,6,7,8-HxCDF	0.1	2,3′,4,4′,5,5′-HxCB (167)	0.00001
1,2,3,4,6,7,8-HpCDF	0.01	2,3,3′,4,4′,5,5′-HpCB (189)	0.0001
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0001		

a) PnCDD, pentachlorodibenzo-*p*-dioxin; HxCDD, hexachlorodibenzo-*p*-dioxin; HpCDD, heptachlorodibenzo-*p*-dioxin; OCDD, octachlorodibenzo-*p*-dioxin; PnCDF, pentachlorodibenzofuran; HxCDF, hexachlorodibenzofuran; HpCDF, heptachlorodibenzofuran; OCDF, octachlorodibenzofuran; TCB, tetrachlorobiphenyl; PnCB, pentachlorobiphenyl; HxCB, hexachlorobiphenyl; HpCB, heptachlorobiphenyl.

PCB performed by the EC Scientific Committee for Food (SCF) [1, 2]. In addition, short summaries are given of the assessments performed by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) [3] and by the US Environmental Protection Agency (US EPA) [4]. There are also a number of other assessments and reviews available on the toxicological properties of dioxins and dioxin-like PCB [5–10]. The assessments focus on TCDD, because this is the only dioxin for which the toxicological database is sufficient to derive a tolerable intake. Following the establishment of a tolerable intake for TCDD, this can be widened to embrace all other, less well-studied, dioxins and dioxin-like PCB by using toxic equivalency factors.

2 Toxic equivalency factors

In almost all matrices, dioxins and PCB are found as complex mixtures. To facilitate the comparison of analytical and exposure data the analytical results are converted into toxic equivalents (TEQ). This conversion is based on the assumption that all 2,3,7,8-substituted PCDD and PCDF, as well as the dioxin-like PCB, bind to the arylhydrocarbon receptor (AhR), and show comparable qualitative effects, but with different potencies. These differences in toxicity are expressed in the toxic equivalency factors (TEF), estimated from the weaker toxicity of the respective congener in relation to the most toxic congener TCDD, which is assigned the arbitrary TEF of 1. The total TEQ value of a sample can be obtained by multiplying the analytically determined amounts of each congener by the corresponding TEF and summing the contribution from each congener, assuming dose additivity. The current TEF scheme for PCDD, PCDF, and dioxin-like PCB for humans (WHO-TEF, Table 1) was proposed by WHO in 1997 [11].

3 Absorption, distribution, biotransformation and excretion

The toxicokinetics of dioxins and PCB depend on their lipophilicity, binding to cytochrome P4501A2 in the liver, and the rate of metabolism. Dioxins with 4, 5, or 6 (tetra-, penta- and hexa-) chorine atoms are well absorbed from the gastrointestinal tract (50–90%, depending on the vehicle) while hepta- and octa-chlorinated congeners are absorbed to a lesser extent [8]. Due to their high lipophilicity and resistance to biotransformation, the dioxins accumulate in the body, mainly in fat tissue and liver. Dioxins pass the placenta of pregnant animals [8] and evidence for transplacental transfer of dioxins in humans has been obtained from analysis of foetal tissues and cord blood samples [1]. In rodents, binding to induced cytochrome P4501A2 has been shown to result in sequestration of dioxin-like compounds in the liver [12]. The major determinant for metabolism is the presence of two adjacent, unsubstituted carbon atoms on the lateral positions [13]. The AhR-dependent cytochrome P4501A1

has been associated with the oxidative metabolism of certain PCDD, PCDF, and PCB congeners [1].

The half-life of 2,3,7,8-TCDD ranged from 12 to 31 days in rats and from 5 to 11 years in humans [1].

4 Mode of action and biochemical effects

The biochemical and toxicological effects of the dioxins are mediated via binding to a specific receptor protein in the cells, the AhR [7, 8]. Upon exposure to a ligand, *e.g.* 2,3,7,8-TCDD, the receptor is translocated from the cytosol into the cell nucleus and the ligand-activated AhR specifically recognises xenobiotic response elements (XRE) in DNA and activates transcription of a battery of dioxin-inducible genes. The physiological function of the AhR in mammals is not fully known, but studies using 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, endocrine, and reproductive systems [1].

Induction of certain drug metabolising enzymes, especially those of the CYP1A family, is a sensitive response to dioxin exposure. A significant induction of ethoxyresorufin-O-deethylase activity has been demonstrated in subchronic studies in rats and mice at dose levels of 0.1 – 0.3 ng 2,3,7,8-TCDD/kg of body weight (bw) per day. The no-observed-effect-level (NOEL) was approximately 0.03 ng/kg bw per day. Induction of CYP1A2-related enzyme activities has been demonstrated at similar doses to that of cytochrome P4501A1 [14 – 16].

Dioxins affect vitamin A levels and a number of growth related parameters, such as the epidermal growth factor and tyrosine kinase activity in a number of tissues. Dioxin exposure has also produced a number of effects on many hormone systems in experimental animals. Dioxin may perturb levels of hormones, the number of hormone receptors, and the serum transport of hormones [7]. Decreases in blood thyroxin, insulin and glucose as well as disruption of the normal feedback mechanisms of the pituitary between plasma levels of testosterone, dihydrotestosterone (DHT) and estradiol and LH secretion have been reported. Effects on the adrenals results in altered levels of circulating glucocorticoids [1].

Some of the effects of dioxins may be associated with induction of oxidative stress. Lipid peroxidation, enhanced DNA single strand breaks, and decreased membrane fluidity has been shown in several tissues after high doses of TCDD. Induction of CYP1A isoforms is associated with oxidative DNA damage and altered metabolism of estradiol leading to the formation of quinoines, and redox cycling has been hypothesised to play a role in the enhanced sensitivity of female rats to TCDD-induced liver tumours [1].

5 Toxicological effects in experimental animals

Lethality after exposure to TCDD shows considerable variation among species, *i. e.* the LD_{50} vary from 1 μg/kg bw in the guinea pig to >1000 μg/kg bw in the hamster. Rats and non-human primates are intermediate in sensitivity. Death follows a characteristic wasting syndrome in which animals mobilise their body fat and muscle mass. Atrophy of the thymus is a characteristic effect of exposure to dioxin. In the rat, acute doses greater than 1 μg/kg bw are required to cause thymic atrophy. Atrophy of the spleen is slightly less sensitive and does not occur in all species. The adult testis and ovary have similar sensitivity to that of the spleen [1].

Liver hyperplasia, fatty infiltration, and necrosis have been observed in a number of species. 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 and proliferative responses are seen at relatively high doses of TCDD [1].

The heart and the entire cardiovascular system appear to be very sensitive targets for TCDD in chicken, resulting in oedema. Recent studies have demonstrated that the heart is also a target in mammals [4, 17, 18]. The gastrointestinal tract, *i. e.* the stomach, undergoes hyperplasia after toxic doses of TCDD in several species, such as non-human primates. Monkeys also respond to TCDD with squamous metaplasia of the meibomian glands on the eyelids, and the ceruminous glands lining the ear canal [1].

The immune system is also a target for TCDD toxicity in multiple animal species and TCDD has caused suppression of both cell-mediated and humoral immunity. In adult animals, these effects have generally been seen at doses that are also associated with thymic atrophy and other overt signs of toxicity. Perinatal exposure of experimental animals to TCDD results in suppression of primarily T cell immune functions, with suppression persisting into adulthood. This suggests that perinatal development is a critical and sensitive period for TCDD-induced immunotoxicity [1, 4].

Reproductive effects have been documented in multiple animal species. High doses are associated with infertility and foetal loss. A multi-generation study in rats reported a no-observed-adverse-effect-level (NOAEL) of 1 ng/kg bw per day for reproductive impairment in the F1 and F2 gen-

erations [19]. The lowest-observed-adverse-effect-level (LOAEL) was 10 ng/kg bw per day. Exposure of rhesus monkeys to approximately 0.8 ng/kg bw per day led to foetal loss due to spontaneous abortions [1]. An increase in the incidence and severity of endometriosis was observed in the female rhesus monkeys administered TCDD in their diet for up to 4 years and held for as long as 10 additional years without treatment [20].

Adverse developmental effects of TCDD, *e. g*. growth retardation, thymic and splenic atrophy, haemorrhage, and oedemas have been observed in foetuses and neonates of many species. Structural malformations, *i. e*. hydronephrosis and cleft palate, have been induced in mice. Prenatal doses of TCDD also accelerated tooth eruption in mice and impaired dentin and enamel formation in continuously growing rat incisors [1].

Prenatal exposure to TCDD results in permanent adverse effects on the developing reproductive, immune, and nervous systems of both male and female offspring in rats [21 – 27]. Male pups demonstrate delayed puberty, altered mating behaviour, and decreased sperm counts. Female pups show genital malformation consisting of vaginal threads and cleft phallus. These effects were all caused by *in utero* exposure at gestation day 15 (GD15) [26, 27]. A series of studies comparing the magnitude of effects of dosing pregnant rats at various time points during the gestation period has indicated that the maternal/foetal tissue concentration at the critical window of sensitivity (day 15 – 16 of gestation) is the key dose metric [28, 29]. Neurobehavioral effects of prenatal exposure to TCDD in rats have resulted in changes in locomotor activity and rearing behaviour, deficits in learning, and hearing deficits in the offspring [1]. The offspring of rhesus monkey dams with a chronic dietary intake of approximately 0.15 ng/kg bw per day for a total of 4 years had deficits in object learning; but improvements in spatial learning [30]. Subtle effects on the developing immune system, such as changes in cell surface markers and a permanent suppression of delayed-type hypersensitivity in the offspring have been observed after prenatal doses lower than those inducing atrophy of the thymus in both mice and rats [1].

A number of experimental studies have shown the carcinogenicity of TCDD. For many years the results of a two-year chronic bioassay performed by Kociba *et al.* in 1978 [31] have formed the primary basis for the safety evaluation of TCDD [8]. In that study, groups of male and female Spartan Sprague-Dawley rats were administered TCDD in their diets at dose levels of 0, 1, 10, and 100 ng 2,3,7,8-TCDD/kg bw per day. The female rat liver was the primary target site with an increased incidence of hyperplastic nodules at 10 and 100 ng/kg bw/day and increase in hepatocellular carcinomas at 100 ng/kg bw per day. Hepatic tumours were not observed in the males. In addition, lung tumours were observed in the females, and the incidence was increased at 100 ng/kg bw per day. The NOAEL for hepatic neoplasms was 1 ng TCDD/kg bw per day. The body burden of TCDD at the NOAEL was 60 ng/kg bw. The carcinogenicity of TCDD has also been assessed in Osborne Mendel rats, where an increased incidence in follicular cell thyroid adenomas was seen in male rats, and hepatocellular carcinomas and adrenal cortical adenomas/carcinomas in female rats at high doses. In addition, the carcinogenicity of TCDD has been demonstrated in male and female mice and in male Syrian Golden hamsters [1].

The US National Toxicology Program (NTP) has recently conducted 2-year lifetime rat studies to evaluate the chronic toxicity and carcinogenicity of TCDD, PCB-126, and 2,3,4,7,8-PeCDF, as well as a mixture of the three compounds. The studies were initiated in order to test the hypothesis of dose-additivity in the carcinogenicity of dioxins. The studies were conducted in female Harlan Sprague-Dawley rats, based on the prior observations of the carcinogenic sensitivity to TCDD in the Spartan Sprague-Dawley rat strain [31]. Doses were established using the current WHO TEF values [11] to provide doses of the individual chemicals or the mixture estimated to be equivalent to those used in the TCDD study (3, 10, 22, 46, 100 ng TCDD/kg bw per day by gavage 5 days per week). The mixture study was designed such that each compound would provide a third of the total dioxin TEQ to the mixture. Treatment and dose-related increases in the incidence of cholangiocarcinoma and hepatocellular adenomas of the liver, cystic karatinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa were seen with all three compounds and the mixture. In addition, the dose response for the mixture could be predicted from a combination of the potency-adjusted doses of the individual compounds, and the results supported the current use of the WHO-TEF approach for dioxin cancer risk assessments [32].

Mutagenicity tests *in vitro* and *in vivo* with TCDD and other dioxins have in general been negative and TCDD does not bind covalently to DNA. Studies in the mouse skin support a lack of initiating activity, but TCDD and other structurally related compounds have shown strong tumour promoting activity for epidermal carcinogenesis in hairless mice, Many studies have also shown that TCDD is a potent tumour-promoting agent in the female rat liver after partial hepatectomy and initiation with diethylnitrosamine (DEN) [1].

6 Human data

6.1 Non-cancer effects

Chloracne is the most widely recognised dermal effect of human exposure to TCDD-contaminated substances. Chloracne has been observed in some workers after all reported accidents at trichlorophenol (TCP) production facilities and among individuals involved in production of TCDD-contaminated products.

Following the Seveso accident in 1976 especially children experienced chloracne; however, the condition disappeared after discontinuation of exposure despite high serum TCDD levels. Increases in serum levels of liver enzymes and of D-glucaric acid excretion in urine were also reported for children in Seveso and among TCP production workers with high serum TCDD levels (>100 pg/g fat) [1]. A change in sex ratio among newborns with an excess of females over males has been described in the period 1977 – 1984 for the most TCDD contaminated area in Seveso [33]. An explanation of this phenomenon has not been offered but a possible role of hormonal disruption cannot be ruled out.

A variety of other end-points has been studied in an extensive number of epidemiological studies involving both occupationally or environmentally exposed cohorts. The majority of these studies have shown inconsistent results and in most cases confounding exposures were not resolved (Table 2) [1].

Multiple, persistent effects were observed among children whose mothers had been exposed during pregnancy to high levels of PCB and PCDF from contaminated rice oil in the Yusho and Yucheng incidents in Japan and Taiwan. The effects included low birth weight, persistent developmental delays throughout childhood and behavioural disorders, hearing loss, and alterations in sexual development. The lowest intake of TEQ estimated to result in minimal Yusho symptoms was 28 ng/kg bw per day for 135 days [1].

Neurodevelopmental delays and neurobehavioral effects have been reported in several cohorts in the USA and Europe. In two cohorts from the USA, the findings were related to high exposure to "total"-PCB in human milk. Dioxins and dioxin-like PCB were not measured. In a study from The Netherlands, *in utero* exposure to dioxins and dioxin-like PCB may have influenced early neurodevelopmental parameters and thyroid hormone status, *e.g.* of thyroxin (T4) and thyroid stimulating hormone (TSH), in infants up to 3 months of age. Transient differences in these parameters were reported when a high exposure group was compared with the low exposure group (below 63 ng total TEQ/kg fat in human milk). The observed differences were subtle, within the normal range and considered to be without clinical relevance [1].In another European study involving also a German cohort, PCB-related delay of mental development was found at 30 months, but upon reassessment between 72 and 77 months developmental delay was no longer observed [34].

Table 2. Human studies on non-cancer effects after occupational or environmental exposure to dioxins [1]

End-point	Outcome
Blood lipids	Inconsistent results
Chloracne	No clear dose-response
Cardiovascular disease	Inconsistent results
Diabetes	Inconsistent findings
Immunological effects	Inconsistent findings
Liver	Increases in liver enzymes
Neurological effects	Inconsistent findings
Neurodevelopmental	Some differences reported
Reproductive hormones	Inconsistent changes
Reproductive outcome	Changes in sex ratio (Seveso)
Respiratory system	Inconsistent evidence
Thyroid function	Small and inconsistent changes
Urinary system	No major changes reported

6.2 Carcinogenicity

In most of the human studies examining the carcinogenicity of dioxins, people were exposed to mixtures of PCDD and TCDD, as contaminants of phenoxy herbicides and chlorophenols. These epidemiological studies have involved subjects with the highest recorded exposures to TCDD. In a large study involving 12 industrial plants in the USA mortality from all cancers was slightly but significantly elevated. Similarly, all-cancer mortality was significantly increased in several studies of workers from The Netherlands and Germany. Excesses at specific sites were reported for urinary bladder, kidney, the digestive system, lung, buccal cavity and pharynx, breast in females, as well as lymphatic and haematopoietic neoplasms and non-Hodgkin lymphoma [1].

The International Agency for Research on Cancer (IARC), in its 1997 re-evaluation of the cancer hazard of dioxin and related compounds, found that although the epidemiologic database for TCDD was still limited, the overall weight of the evidence provided by human, animal and mechanistic data was sufficient to characterize TCDD as a human carcinogen (Group 1). The other, related dioxin-like compounds were considered not classifiable as to its carcinogenicity to humans (Group 3) [6].

7 The SCF risk assessments of PCDD, PCDF, and dioxin-like PCB in food

In its risk assessments of dioxins and dioxin-like PCB in food the SCF [1, 2] took notice of a previous evaluation of

dioxins and dioxin-like PCB carried out by a WHO Consultation in 1998 [35]. At that meeting, a tolerable daily intake (TDI) for humans was suggested as a range of 1–4 pg WHO-TEQ/kg bw for dioxins and dioxin-like PCB. In agreement with the WHO Consultation the SCF concluded that (i) due to the accumulative nature of dioxins, body burdens should be used as dose metrics rather than the daily dose in order to compare across species, (ii) the available studies in humans were not suitable for use in the risk assessment, and (iii) the evaluation should ultimately be based on the most sensitive adverse effects seen in experimental animals, which were developmental, reproductive and hormonal effects in the offspring of dams administered TCDD during pregnancy, *i. e*. these were the adverse effects occurring at the lowest body burdens. Thus, the assessments deviated from earlier assessments of TCDD by not using the liver toxicity and carcinogenicity reported in long-term rat studies as the most critical endpoints. As TCDD is not a direct-acting genotoxic agent, the SCF, in contrast to the US EPA [4], found that a threshold could be assumed for the carcinogenic effect and moreover, carcinogenicity was not considered the most sensitive adverse effect of TCDD.

7.1 Use of body burden as dose metric

Due to the large differences in TCDD half-lives across species, ranging from 20 to 30 days in rats, over approximately 400 days in monkeys, and between 5 and 11 years in humans, rodent species require appreciably higher doses (100 to 200-fold) to reach the same equivalent body burdens at steady state as recorded in humans at background exposures. The most appropriate dose metric would ideally be the concentration at the target tissue. However, it is seldom known. DeVito *et al.* [36] found that for a number of effects, humans and animals respond to TCDD at similar body burdens. Therefore, the body burden of TCDD in the experimental animals was considered the most appropriate surrogate to be used as the dose metric.

In order to transform animal body burdens into the equivalent estimated human daily intakes (EHDI) that on a chronic basis would lead to similar body burdens in humans (at steady state) simple, classical pharmacokinetic calculations were used. The elimination of dioxins at low doses follows first-order kinetics and is independent of the body burden or dose. The relation between the total steady state body burden and intake is:

$$\text{Body Burden at steady state (ng/kg bw)} = f \times \text{Intake (ng/kg bw/day)} \times \text{half-life in days}/\ln(2) \quad (1)$$

where f is the fraction of dose absorbed (assumed to be 50% for absorption from food for humans), and an estimated half-life for TCDD of 2740 days (7.5 years). For compounds following first order kinetics it will take 3–4 half-lives to approach steady state. For dioxins, this is equivalent to 20–30 years [1].

7.2 The assessment by SCF in 2000 [1]

The most sensitive adverse effects reported for TCDD were developmental and reproductive effects in rats and monkeys and an increase in the incidence of endometriosis in monkeys. For most of these effects, NOAEL were not identified in the studies available, only LOAEL. The TCDD body burdens of the experimental animals in these studies were estimated and the associated EHDI were calculated using the Eq. (1) given above (Table 3).

In one study, chronic dietary exposure of female rhesus monkeys at 5 ng TCDD/kg of diet (0.15 ng TCDD/kg

Table 3. Estimated animal body burdens of 2,3,7,8-TCDD and associated EHDI [1]

Study	Response at LOAEL	LOAEL	Maternal body burden (ng/kg bw)[a)]	Associated EHDI (pg/kg bw)
[30]	Rhesus monkeys: Subtle, non-persistent neurobehavioral changes (object learning) in offspring	0.15 ng/kg bw/day dietary administration	25–37[b)]	12.5–18.5
[19]	Rhesus monkeys: Endometriosis	0.15 ng/kg bw/day dietary administration	39[c)]	19.5
[26]	Long Evans rats: Accelerated eye opening and decreased sperm count in male offspring	50 ng/kg bw single bolus dose by gavage	30[d)]	15
[23]	Holzman rats: Decreased sperm count in offspring	64 ng/kg bw single bolus dose by gavage	38[d)]	19
[37, 38]	F344 rats: Immune suppression in offspring	100 ng/kg bw single bolus dose by gavage	60[d)]	30

a) Increment over background. Background body burden in rats and mice is about 4 ng TEQ/kg bw [35].
b) Maternal body burden at delivery after 16.2 and 36.3 months of maternal exposure, respectively.
c) Body burden at the end of the dosing period (42 months).
d) Maternal body burden at gestational day 15.

bw/day) produced a subtle change in one, among several, parameters related to cognitive recognition (object learning) in offspring delivered after means of 16.2 or 36.3 months of maternal exposure [30]. The SCF was of the opinion that this subtle, non-persistent change was of doubtful significance for humans.

Female rhesus monkeys fed a diet containing 5 or 25 ng TCDD per kg for 3.5 or 4 years, respectively, developed higher incidences of endometriosis than control monkeys, when the animals were followed for up to ten more years without additional TCDD exposure. The severity of the disease was correlated with the cumulative TCDD exposure [19].

There was also sufficient evidence that prenatal TCDD exposure of rodents produces a number of adverse effects on the developing male and female reproductive organs and their functions. It was found that 50 ng TCDD/kg bw administered by gavage as a single bolus dose to pregnant Long Evans rats at GD15 represents a sensitive LOAEL in producing accelerated eye opening and a non-significant decrease in sperm counts in the male offspring [26], while 64 ng TCDD/kg bw produced a significant reduction in ejaculated sperm count in the offspring of Holzman rats [23]. Clear effects on several other parameters of male reproductive function, morphology and behaviour were seen in the Holzman rat at a dose level of 160 ng TCDD/kg bw [21 – 23].

A study using pregnant F344 rats has demonstrated that prenatal exposure to a single bolus dose of 100 ng TCDD/kg bw on GD14 produced slight but significant suppression of delayed-type hypersensitivity in the male offspring of F344 rats at 14 months of age. Thus, the LOAEL for delayed type hypersensitivity suppression in male offspring following prenatal exposure was 0.1 μg TCDD/kg bw [37, 38].

The bioavailability of TCDD was not measured in any of the studies from which the LOAEL were derived. However, from a study by Hurst *et al*. [39] it was concluded that 60% would be an appropriate estimate for the bioavailability of TCDD in pregnant rats at the dose levels associated with the LOAEL. As regarding absorption from the diet, the SCF assumed absorption of 50% from food for humans [35]. Based on these assumptions the SCF calculated that these sensitive adverse responses were associated with exposure levels that produced maternal body burdens between 25 and 60 ng TCDD/kg bw. The associated EHDI were in the range of 12.5 – 30 pg TCDD/kg bw.

In order to arrive at a tolerable intake of TCDD for humans an uncertainty factor has to be applied. The uncertainty factor should account for the use of LOAEL instead of a NOAEL, the possible differences between experimental animals and humans in susceptibility (toxicokinetics and toxicodynamics) to TCDD and the potential interindividual variation in susceptibility (toxicokinetics and toxicodynamics) to TCDD within the human population.

The LOAEL reported for the sensitive endpoints were considered to be close to the NOAEL and representing marginal effects. The SCF found it therefore appropriate to use a factor of only 3 to allow extrapolation to NOAEL. The use of an uncertainty factor to account for differences between experimental animals and humans in toxicokinetics was not required since body burdens had been used to scale doses across species. The SCF used the default uncertainty factor of 3.2 as recommended by WHO [40] to account for interindividual variations in toxicokinetics of TCDD within the human population. In addition, the SCF agreed with WHO [35] in considering that humans are not more sensitive to TCDD than responsive rodent strains. Therefore, no uncertainty factor was applied for differences in toxicodynamics between experimental animals and humans and for interindividual variation among humans. Overall an uncertainty factor of 10 (3×3.2) was considered adequate for the protection of human health from exposure to TCDD.

Applying the 10-fold uncertainty factor to the EHDI in Table 3 suggested a tolerable intake in the range 1 to 3 (rounded figures) pg/kg bw per day. In recognising that compounds like TCDD have very long half-lives in the human body, it was found more appropriate to express the tolerable intake on a weekly basis. Therefore a temporary tolerable weekly intake (t-TWI) of 7 pg TCDD/kg bw was established and was extended to include all 2,3,7,8-substituted PCDD and PCDF, and the dioxin-like PCB, expressed as WHO TEQ.

7.3 The SCF 2001 update of its risk assessment of dioxins and dioxin-like PCB [2]

The major problem in the assessment from 2000 was related to the bioavailability of TCDD to the foetus at a given maternal body burden because this may differ between a bolus dose (as in the rat studies used) 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 TCDD levels would be elevated before redistribution to the tissue compartments. In contrast, low-level chronic exposure will not significantly elevate serum levels. The critical determinant of these reproductive effects is the foetal concentration around GD 15 – 16 [28, 29], which is likely to be higher following a single bolus dose on GD 15 than that resulting from lower level chronic exposure. This weakened the relevance of the assessment to human dietary exposure [2].

Table 4. Comparison of average maternal and foetal body burdens after single dose and subchronic TCDD exposures to pregnant rats [2]

Single dose exposure at GD 15[a]				Subchronic exposure[b]			
Single dose[c]	Body burden measured at GD 16			Adjusted daily dose[d]	Body burden measured at GD 16		
	Maternal[c]	Foetal[c]	Maternal/ Foetal		Maternal[c]	Foetal[c]	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				

a) Data from [39].
b) Data from [41].
c) ng/kg bw.
d) ng/kg bw per day, adjusted to continuous exposure from 5 days/week.

Table 5. Corresponding values of modelled foetal, acute maternal and subchronic steady state maternal body burdens of 2,3,7,8-TCDD [2]

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
1.4	5.9	14.6	2.5
1.7	7.5	18.6	2.5
1.8	8.0	20.0	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
7.5	47.5	122	2.6
8.0	52	134	2.6
9.0	60	156	2.6
13.2	95.7	251	2.6
15.2	113	299	2.7

The key to the use of the rat data for risk assessment came with a new study by Hurst *et al*. [41]. Thus, in two studies the tissue distributions of ^{3}H in maternal and foetal tissue on GD16 were reported either after administration of [^{3}H]-TCDD as a single dose on GD15 or following subchronic exposure (5 days per week for 13 weeks) [39, 41] (Table 4). The SCF analysed these data and performed a best-fit analysis of each data set in the range of foetal body burdens from 0 to 15.2 ng/kg bw with the curves constrained to pass through the origin. It was found that both data sets could be fit to power equations. These equations were used to calculate the corresponding acute and subchronic maternal body burdens for a number of foetal body burdens. From these calculations a correction factor of 2.5 could be derived for maternal body burdens of <30 ng/kg bw and 2.6 from 30 – 100 ng/kg bw for going from single gavage dose body burdens to dietary steady state body burdens (Table 5) [2].

The SCF reconsidered the rat studies used in its first assessment in the light of the new information (Table 6). The studies on developmental immunotoxicity were not used again because this effect required higher body burdens than the effects on the male reproductive organs. In addition, new information [42, 43] had become available, which resulted in uncertainties about the relevance of the monkey studies for the risk assessment. They were therefore not considered further by the SCF in the update [2].

Instead, two additional toxicological studies using rats were included (Table 6). In a study by Faqi *et al*. [44], Wistar dams received an initial intramuscular loading dose of 25, 60, or 300 ng TCDD/kg bw 2 weeks prior to mating, followed by weekly maintenance doses of 5, 12, or 60 ng TCDD/kg bw. The size of the maintenance doses was based on an elimination half-life of 3 weeks for adult rats. After birth, developmental landmarks in the male offspring were monitored. The number of sperm per cauda epididymis and daily sperm production was reduced in all TCDD treated groups at puberty and at adulthood. The intended maternal steady state body burden at the LOAEL in this study was 25 ng TCDD/kg bw. The SCF estimated the corresponding total foetal body burden at 3.0 ng/kg bw. According to Table 5, a maternal body burden of 39 ng TCDD/kg bw at steady state would be needed to produce this foetal body burden (Table 6).

In a study by Ohsako *et al.* [45] pregnant Holzman rats were given a single oral dose of 0, 12.5, 50, 200 or 800 ng TCDD/kg bw on GD 15, and the male offspring were examined on postnatal day 49 or 120. In this study, there were no changes seen neither on testicular or epididymal weights nor in daily sperm production or sperm reserve. However, the anogenital distance of male rats sacrificed on postnatal day 120 showed a significant decrease in the groups receiving doses of 50 ng TCDD/kg or higher. Assuming that 60% of a single gavage dose at the NOAEL of 12.5 ng TCDD/kg

Table 6. Estimated animal steady state body burdens of 2,3,7,8-TCDD and associated EHDI at NOAEL and LOAEL in the pivotal studies [2]

Study	Endpoint	NOAEL	LOAEL	Estimated maternal steady state body burden[a] (ng/kg bw)	Associated EHDI (pg/kg bw)
Mably *et al.*, 1992 [23]	Holzman rats: Decreased sperm count in male offspring		64 ng/kg bw single bolus dose by gavage	99[b]	49.5
Gray *et al.*, 1997 [26]	Long Evans rats: Accelerated eye opening and decreased sperm count in male offspring		50 ng/kg bw single bolus dose by gavage	79[b]	39.5
Faqi *et al*., 1998 [44]	Wistar rats: Decreased sperm production and altered sexual behaviour in male offspring		Maintenance of 25 ng/kg bw by subcutaneous injections	39[b]	19.5
Ohsako *et al.,* 2001 [45]	Holzman rats: Decreased anogenital distance in male offspring	12.5 ng/kg bw single bolus dose by gavage		19[c]	9.5
			50 ng/kg bw single bolus dose by gavage	79[c]	39.5

a) Increment over background. Background body burden in rats is about 4 ng TEQ/kg bw [35].
b) Composite value resulting from of pseudo steady state body burden and acute body burden on GD 15.
c) Maternal body burden at gestation day 16.

bw was retained in the body at GD 16 this 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 TCDD administration. The LOAEL level of 50 ng TCDD/kg bw corresponds to a maternal body burden of 30 ng/kg bw, which would equate to a steady state maternal body burden of 79 ng TCDD/kg bw (Table 6).

Applying a 3.2-fold uncertainty factor to the EHDI of 9.5 pg TCDD/kg bw, calculated from the NOAEL in the Ohsako *et al.* study [45], a tolerable intake of 3 pg/kg bw per day was derived. However, when the lowest LOAEL was used instead of the NOAEL, an additional uncertainty factor of 3 was needed and in applying a 9.6-fold overall uncertainty factor to the EHDI of 19.5 pg/kg bw calculated from the LOAEL in the study of Faqi *et al*. [44] a tolerable intake of 2 pg/kg bw per day was derived. Using a similar approach to the LOAEL in the other studies in Table 6 would result in 4.1 and 5.2 pg/kg bw per day for the tolerable intake [2].

In line with the previous evaluation by the SCF, the lowest tolerable intake of 2 pg TCDD/kg bw/day was expressed as a group tolerable weekly intake of 14 pg WHO-TEQ/kg bw [2].

7.4 The SCF risk characterisation

The average dietary intakes of PCDD and PCDF for adults of various European countries were estimated to be between 0.4 – 1.5 pg TEQ/kg bw per day, and the average dietary intakes of dioxin-like PCBs were estimated as being 0.8 – 1.5 pg PCB-TEQ/kg bw per day [1]. Thus, the total intake of dioxins and dioxin-like PCB from the diet was equivalent to 1.2 to 3.0 pg WHO TEQ/kg bw/day. From these intake estimates it is evident that a considerable proportion of the European population would exceed the group TWI of 14 pg WHO-TEQ/kg bw derived by the SCF.

During the nursing period, breast-fed infants may have intakes of these compounds on a body weight basis estimated to be 1 to 2 orders of magnitude higher than the average adult intake. In this context, several WHO meetings on the health significance of contamination of human milk with dioxins and PCB have concluded that the available evidence from studies in humans does not justify altering recommendations on the promotion of, and support for, breast-feeding [1]

8 Other recent risk assessments of PCDD, PCDF, and dioxin-like PCB

8.1 The JECFA 2001 evaluation

In June 2001, JECFA established a provisional tolerable monthly intake (PTMI) of 70 pg WHO-TEQ/kg bw (equivalent to 2.33 pg TEQ/kg bw per day) for PCDD, PCDF, and dioxin-like PCB [3]. JECFA, like the SCF, treated dioxins as nongenotoxic carcinogens and assumed that a safety factor approach, based on noncancer effects observed at lower body burdens than cancer in animals and human, would be adequate to account for concerns for both cancer and noncancer effects. JECFA therefore used an approach essentially similar to that used by the SCF in its

2001 reassessment [2]. The same pivotal studies and safety factors were used, but JECFA used two different models to extrapolate the maternal body burden at the NOEL/LOEL of the pivotal studies. The Committee then chose the PTMI as the mid-point of the range of values from its analysis [3].

JECFA quotes intakes estimated from national food consumption data to be 33 – 42 pg/kg bw per month at the median and 81 – 100 pg/kg bw per month at the 90th percentile for PCDD and PCDF, and 9 – 47 pg/kg bw per month at the median and 25 – 130 pg/kg bw per month at the 90th percentile for dioxin-like PCB. Estimates could not be made for the sum of PCDD, PCDF, and dioxin-like PCB, because countries submitted data on concentrations separately. However, from these intake estimates it is also evident that a considerable proportion of these populations would exceed the PTMI of 70 pg WHO-TEQ/kg bw derived by JECFA [3].

8.2 The US EPA draft human health reassessment of TCDD and related compounds

The US EPA draft human health reassessment of TCDD and related compounds, dated December 2003 [4], used a somewhat different approach than the assessments by WHO [35], SCF [1, 2] and JECFA [3] in paying special attention to the studies on carcinogenic effects of dioxins in humans and animals and by using a margin of exposure (MOE) approach for the noncarcinogenic effects seen in animal studies instead of establishing a tolerable intake. The EPA agreed with the previous assessments that the similarities in toxicity, both cancer and noncancer, between species and across different dioxin congeners is due to a common mode of action via initial binding to the AhR and that the use of the toxic equivalency factor (WHO-TEF) concept to sum the contributions of individual PCDD, PCDF, and coplanar PCB congeners with dioxin-like activity is a valid approach. The EPA also considered the use of integrated measures of dose such as lifetime average body burden, or body burden during a special window of sensitivity, as more appropriate default dose metrics than average daily intakes.

For cancer outcomes, EPA was of the opinion that the epidemiological evidence shows consistent statistically significant elevations, with dose-response trends, for all cancers combined and lung cancer risk in occupational cohorts. In addition, there was evidence of possible additional elevations in tissue-specific cancer rates. Together with the positive animal cancer studies and mechanistic considerations, the EPA characterized TCDD as "carcinogenic to humans". In addition, based on similarities of response in animal studies for non-TCDD congeners and mixtures, mode of action studies, and consistent with the concept of toxic equivalency, EPA considered complex mixtures of dioxin and related compounds as highly potent "likely" carcinogens.

The EPA calculated the body burdens of dioxin and dioxin-like substances leading to an estimated 1% increase (ED_{01}) in the lifetime risk of cancer in the three occupational studies with the best exposure information. The ED_{01} for all cancers combined from the three occupational cohorts ranged from 6 to 62 ng TCDD/kg bw, depending on the study and the model used, and those calculated from the animal data fell in the middle of this range. By comparison, background body burdens in the United States were estimated to be approximately 5 ng TEQ/kg bw, suggesting a small margin of exposure.

From these same occupational and animal cancer studies, EPA estimated an upper bound on the lifetime risk of all cancers combined of 1×10^{-3} per pg WHO-TEQ/kg bw per day as an estimator of cancer risk for both background intakes and incremental intakes above background. This risk estimate was based on the assumptions that the extra cancer risk seen in the occupational cohorts is attributable to dioxin and not other chemical agents present, that the appropriate metric for cancer risk is lifetime average body burden, and that the dose-response model curve continues linearly below the range of statistically significant data. Using the upper-bound risk estimate means that there is greater than a 95% chance that "true" population cancer risks will be less than the upper bound estimate.

For the pivotal noncancer hazards (developmental/reproductive toxicity, developmental neurobehavioral and neurochemical alterations, endocrine effects, and developmental immunotoxicity) the EPA did not establish an RfD value (equivalent to a tolerable intake), which would be a normal procedure. EPA argues that in contrast to WHO, SCF, and JECFA, who used composite uncertainty factors at about 10, the traditional EPA approach would yield a composite uncertainty factor in the range of 30 to 100 or even more. Applying such standard procedures to the pivotal noncancer data would result in an RfD below the current estimated average intake by the American population (~1 pg WHO-TEQ/kg bw per day), and would therefore be uninformative for a safety assessment. Instead, the EPA chose to characterize the margin of exposures (MOE). The MOE is the ratio of the effect level in the comparison species (ED_{01} or LOEL) to the human body burden. For the most sensitive endpoints identified, MOE ranged from less than 1 for enzyme induction in mice and rats, 4 for developmental neurotoxicity and endometriosis in non-human primates, 5 – 8 for developmental/reproductive toxicity in rats, and 12 for developmental immunotoxicity in rats. In evaluating the MOE, consideration should be given to uncertainties in dis-

tinguishing between adaptive biochemical changes and adverse effects.

The EPA also considered that foetuses, infants, and children are exposed to dioxins through several routes. The foetus is exposed *in utero* to levels of dioxin and related compounds that reflect the body burden of the mother. However, it is important to recognize that the greatest impact on the mother's body burden is from her lifetime exposure history rather than from the individual meals she eats during pregnancy. Nursing infants represent special cases because for a limited, but developmental important, portion of their lives they may have elevated exposures on a body-weight basis when compared with non-nursing infants and with adults. However, the EPA considered that the benefits of breast-feeding are widely recognized to outweigh the risks.

9 Conclusions

From these risk assessments, irrespective of it having been performed by WHO, SCF, JECFA, or US EPA, it is evident that PCDD, PCDF, and dioxin-like PCB can produce effects at or near current background human body burdens or intake levels. Some of the effects are indicative of a biological response to dioxin exposure and some are clearly adverse. Therefore, given the obvious uncertainties in these risk assessments continuing efforts to lower human exposure by controlling releases to the environment and hence accumulations in the food chains are warranted.

10 References

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