



Summary Risk Assessment

CASCADE Model Compounds – TCDD

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Introduction

The present document is a deliverable of WP10 of CASCADE. This summary is a deliverable of WP10 of CASCADE. The document aims to summarize available health risk assessments and evaluations for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The focus of the document is on consumers and the possible health risks that consumers may be challenged to when exposed to TCDD via food commodities.

Additional aims of the present document are to identify data gaps and research needs to further improve the risk assessment of TCDD, to identify and integrate novel information generated within the CASCADE network as well as in the open literature that can be used in the data-base for future risk assessments, and to use the information of the document for further comparative risk assessment methodology work.

An extended version of this document is being revised and will be available at a later date.



Available Risk Assessments

Comprehensive risk assessments of TCDD have been performed by a number of international organisations including the International Agency for Research on Cancer (IARC), the WHO European Centre for Environmental Health (WHO-ECEH), the EU Scientific Committee on Food (EU-SCF), the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In addition, several national regulatory agencies have established human exposure standards, including the US-ATSDR, the US EPA, Japan, the Netherlands, Germany, the Nordic countries, UK and Canada. There is also a published assessment from Exponent, a major US consulting firm. (These risk assessments are summarized in Appendix 1)

In 1997, the IARC classified TCDD (but not other dioxin-like compounds) as “carcinogenic to humans” (Group 1) (IARC 1997). The original decision was based on evidence in four groups of highly exposed industrial workers and the fact that TCDD is shown to be a multisite carcinogen in several animal species and the similarity of the AhR mechanism between humans and animals. The estimated blood lipid levels of the exposed workers were also found to be similar to the concentrations of TCDD in rats exposed to carcinogenic dosage regimens. Since the IARC classification in 1997 there is new epidemiologic evidence in several of the industrial cohorts, as well as evidence of excesses from several specific cancers in the Seveso accident cohort. There are also new data regarding how the Ah receptor functions in mediating the carcinogenic response to TCDD that supports the 1997 IARC classification (Steenland *et al.*, 2004). The decision by IARC has been criticised for not taking the large inter-species variation into account, for the use of mechanistic considerations in stead of sufficient evidence in humans, for not evaluating confounding lifestyle factors, such as smoking, for underestimating the tissue concentrations of highly exposed workers, for the fact that there are few precedents of carcinogens that increase cancer at all sites without excess of any specific tumour. In addition, the decision to categorize other dioxin-like compounds as “not classifiable” (Group 3) has been criticised.

One of the main paradigm shifts in risk assessment of dioxin-like compounds was made during the WHO-ECEH consultation in 1998 when body burden for the first time was used instead of intake (van Leeuwen and Younes 2000). Human daily intakes that would lead to body burdens similar to those associated with adverse effects in animals were estimated to be in the range 14-37 pg/kg/day. The most sensitive adverse effects were found in offspring to exposed dams: Decreased sperm count in rat (Mably *et al.*, 1992; Gray *et al.*, 1997), Immune suppression in rats (Gehrs *et al.*, 1997) (Gehrs and Smialowicz 1998), Genital malformations in rats (Gray *et al.*, 1997; Gehrs and Smialowicz 1998), neurobehavioral effects in monkeys (Schantz and Bowman 1989). One study on endometriosis in exposed monkeys (Rier *et al.*, 1993) was also considered. By applying an uncertainty factor of 10 (a factor 2-3 for extrapolation from LOAEL to NOAEL and a factor 3-5 for dynamic differences between human individuals) a range of 1-4 pg TEQ/kg/day was established as a Tolerable *Daily Intake* (TDI). The upper range should be considered as a maximal tolerable intake on a provisional basis and the ultimate goal is to reduce human intake levels below 1 pg/kg/day. The use of a range value may be toxicologically sound, but have been criticised for being difficult to communicate and the uncertainty margin between the most sensitive end point (14 pg/kg/d)



and the upper TDI-value is only 3.5. It has been discussed whether or not the factor of ten is large enough to compensate for the use of LOAEL, extrapolation between animals and humans in susceptibility, extrapolation within the human population, and kinetic differences between congeners.

Using almost the same pivotal studies as WHO, but with some more recent data on developmental toxicology (Gehrs and Smialowicz 1999), the EU-SCF performed a risk assessment in November 2000 (SCF 2000). The group of experts decided on a Tolerable *Weekly Intake* (TWI) of 7 pg/kg. Six month later, after criticism from the Swedish and Norwegian Food Administrations, as well as from some members (though without personal reference) of the Scientific Committee on Toxicology, the EU-SCF carried out a re-evaluation (SCF 2001). At this time, the study on immune suppression (Gehrs and Smialowicz 1999) was dismissed after considering data on the relation between fetal body burden and maternal body burden (Hurst *et al.*, 2000a and b). These studies indicated that, to reach a certain fetal body burden the maternal body burden after subchronic exposure had to be about 2.5 times the body burden after acute exposure. In the re-evaluation of the neurobehavioral studies on monkeys, the committee was not anymore convinced that this data had clinical significance for humans. The endometriosis-study was criticized for problems with reporting and methods. The fact that the exposed monkeys had higher serum values of other dioxin-like compounds (than the tested, TCDD) than the control animals was considered (Rier *et al.*, 2001). As new pivotal studies the SCF identified a rat study on decreased anogenital distance (Ohsako *et al.*, 2001) and a rat study on neurobehavioral effects and sperm production (Faqi *et al.*, 1998). The change of pivotal studies resulted in a shift to LOAELs ranging between 40 and 100 ng/kg. In addition, a NOAEL value of 20 ng/kg was identified (Ohsako *et al.*, 2001). Using the same estimates on body burden as WHO, and an uncertainty factor of 9.6 (3 for the use of LOAEL and 3.2 for the extrapolation between animals and humans as well as between humans) a TWI of 14 pg/kg was decided. The EU-SCF noted that the TWI would adequately protect against the carcinogenic effect of TCDD, which require higher body burdens and for which a threshold approach is applicable due to its non-genotoxic nature.

The JECFA (2001) evaluated TCDD at its meeting in June 2001 (JECFA, 2001). The meeting concluded that the LOAEL established by Faqi and co-workers (Faqi *et al.*, 1998) and the NOAEL provided by Ohsako *et al.* (2001) could be used to establish a tolerable intake level. Two kinetic models were used to estimate the equivalent maternal body burden with long-term dosing from fetal body burden. After compensating for the background body burden found in untreated rats, JECFA derived a human monthly intake estimate of between 237 and 630 pg/kg. After applying uncertainty factors of 9.6 (3 for the use of LOAEL and 3.2 for the extrapolation between animals and humans as well as between humans) a Tolerable *Monthly Intakes* (TMI) of between 44 and 103 pg/kg was calculated. The mean-point of the range (70 pg/kg) was decided as TMI value.

The US-ATSDR estimated a Minimal Risk Level (MRL) of 1 pg/kg/day for chronic (>365 days) oral exposure to TCDD (ATSDR 1998). As a pivotal study, they use developmental toxicity in monkeys observed in offspring of dams exposed to 5 ppt (= pg/g) TCDD in the diet, corresponding to a daily intake of 120 pg/kg (Schantz *et al.*, 1992). An uncertainty factor



of 90 was used (3 for the use of LOAEL, 3 for the extrapolation from animals to humans, and 10 for human variability) to derive the MRL. The US-ATDSR also derive a MRL of 200 pg/kg/d for acute-duration (<14 days) based on immunological effects in female mice (Burleson *et al.*, 1996). An uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) and a modifying factor of 0,7 to compensate the high bioavailability from an oily vehicle. An intermediate duration (15-364 days) MRL of 20 pg/kg/day was also derived, based on decreased thymus weight in guinea pigs (DeCaprio *et al.*, 1986). An uncertainty factor of 30 (3 for interspecies variation and 10 for human variability) was used.

The US-EPA initiated the work to re-assess the risks posed by TCDD in 1991. In a draft (US-EPA, 2004) the US-EPA did not recommend a reference dose (RfD), reasoning that any estimated value using their standard risk assessment methodology, would be 100-1000 times lower than current human intake. The decision of not recommending a RfD has been criticised for being inconsistent.

Other national regulatory agencies that have established human exposure standards (*e.g.* TDI) range from 1 pg/kg/day (the Netherlands (Netherlands, 1996) and Germany (Umweltbundesamt, 2002)), through 2 pg/kg/d (UK) (COT 2001), 2.3 pg/kg/day (Australia)(Australia, 2004), 4 pg/kg/day (Japan)(J-EPA 1999), 5 pg/kg/day (Nordic countries)(Ahlborg *et al.*, 1988; Johansson and Hanberg 2000), to 10 pg/kg/day (Environment, 1997)(Canada).

Data Gaps and Research Needs

From the available risk assessments several data gaps were identified regarding missing data that would improve the accuracy/precision of the TDI calculation. The research needs are related to mainly five main areas; effect studies, epidemiology studies, dose-response modelling, kinetic models and the TEF concept.

Effect studies

There is a special need for knowledge about effects, that were considered important, but where current data were not sufficient to be used for TDI calculations.

- Immunotoxicity. Better dose-response data in animals, epidemiological studies regarding endpoints such as allergy, vaccination response etc.
- Endometriosis. Further epidemiological studies needed as well as mechanistic studies to confirm causality.
- Bone and teeth effects. Further studies are needed to explore dose-response relationships.
- Endocrine and reproductive effects. Research on effects in animals exposed early after birth. Epidemiological studies in children and adolescents.
- Fertility. Epidemiological studies are required although difficult to design.



- Cardiovascular disease. Animal studies are needed to elucidate if the effects on the heart and circulatory system, seen in a few experimental studies, may cause adverse health effects.
- Diabetes. Prospective epidemiological studies are needed in order to confirm the associations observed in retrospective studies.

For all the effects mentioned above, studies of low-dose effects and dose-response relationships are important. Also for all sensitive effects mechanistic studies are required in order to reduce uncertainty in extrapolation of risk from animals to the most sensitive human individuals. This is important for example in the case of cancer.

Epidemiological studies

As mentioned above there is a lack of knowledge about possible human effects after childhood/adolescence exposure. Further epidemiological studies of these age groups in highly exposed cohorts (Seveso, Yusho, etc.) are important. Moreover, comparative studies of sensitivity in prenatal, adolescent and adult stages are needed.

Dose-response assessment

Further refinement of the BMD methodology is needed. Important aspects include curve fitting in the low-dose area, comparison between endpoints etc.

Kinetic models

The kinetic aspects are very important in dioxin risk assessment. Research that would help to improve the risk assessment include kinetic modelling of groups of the population (probabilistic modelling), the influence of body fat content on kinetics and extrapolation between animals and humans, and half-lives of congeners other than TCDD. For example, are different (higher) half-lives for important congeners accounted for in the TEF-scheme?

TEF-concept

Beside these four areas we conclude that the TEF-scheme is very critical in the risk assessment and risk management of dioxins. Thus, the TEF-concept as well as the individual TEF values, which have several limitations, should be refined as far as possible. Important aspects include appropriate basal studies, refined calculations of REP and TEF, preferably using BMD methodology.



Appendix 1. A summary of all the available risk assessments and the toxicological evaluation of dioxins from 1998 to 2004 concerning the general consumer (oral exposure).

Year	Risk Assessment	Tox data Reference last entry	Critical study	Critical effects/study	Threshold values (ng/kg/day)		Guidance values		
					NOAEL ¹	LOAEL ²	TDI ³	MRL ⁴	MOE ⁵
1998	WHO-ECEH	1998	Rat single oral ⁶ Rat single oral ⁷ Monkey chronic oral ⁸ Monkey chronic oral ⁹	Reproductive toxicity Immunotoxicity Developmental effects Endometriosis		64/200* 100* 0.16 0.16	1-4 ¹⁰		
1998	ATSDR	1998	Monkey chronic oral ¹¹	Developmental effects		0.12		1 ¹²	
2001	SCF ¹³	2001	Rat chronic s.c. ¹⁴	Developmental effects		25	2 ¹⁵		
2001	JECFA	2001	Rat chronic s.c. ¹⁴ Rat single oral ¹⁶	Developmental effects Reproductive effects	12.5*	25	2.3 ¹⁷		
2004	US-EPA ¹⁸	2004	Monkey chronic oral ¹¹ Monkey chronic oral ⁹	Developmental effects Hormonal effects		5** 5**			≤4 4

* Note: single doses (ng/kg)

** Note: ppt (ng/kg)



¹ NOAEL= No Observed Adverse Effect Level

² LOAEL= Lowest Observed Adverse Effect Level

³ TDI= Tolerable Daily Intake (pg/kg/day)

⁴ MRL= Minimal Risk Level (pg/kg/day)

⁵ MOE= Margin of Exposure (LED_{10/01}/environmental exposure of interest) (LED= lower limit on effective dose₀₁)

⁶ Gray et al., 1997a/b: A reproductive toxicity study of male and female Long Evans Hooded rat offspring. Dams were administered a single dose of TCDD on gestational day 15. No guidelines mentioned. a) Significantly decreased cauda epididymal and ejaculated sperm counts were observed in male offspring, LOAEL 64 ng/kg. b) Increased genital malformations, such as vaginal threads, in a dose related fashion were observed in the female offspring, LOAEL 200 ng/kg.

⁷ Gehrs et al., 1997b: An immunotoxicity study of F344 rats after exposure to TCDD. Dams were orally administered a single dose of TCDD on gestational day 14. No guidelines are mentioned in the article. Immune suppression was observed in the offspring; decreased percentage of thymocytes, splenocytes, thymic cellularity and a suppressed delayed-type hypersensitivity (DTH) response was observed in both males and females, lymphoproliferative responses of splenocytes to pokeweed mitogen (PWM) was significantly suppressed in female offspring.

⁸ Schantz and Bowman, 1989: Developmental toxicity study in Rhesus monkeys exposed pre-natally to TCDD through the diet. No guidelines mentioned. In tests of cognitive function, object learning was significantly impaired in the offspring, but no effect on spatial learning was observed LOAEL 5ppt.

⁹ Rier et al., 1993: Endometriosis study in Rhesus monkeys (macaca mulatta) chronically exposed to TCDD through the diet. Guidelines not mentioned. An increased incidence in the development of endometriosis was observed in the monkeys and significantly correlated with the exposure dose of TCDD, LOAEL 5ppt.

¹⁰ The TDI is based on the estimated daily intakes (EDI) in humans of 14-37 pg/kg/day and is related to effects following acute gavage (bolus) exposure in rats to effects seen after a dietary exposure in monkeys for a prolonged period of time (4 years), the latter resembles more the conditions of human intake of these compounds. The TDI of 1-4 TEQ pg/kg bw/day was established by applying an uncertainty factor of 10 to the EDI.

¹¹ Schantz et al., 1992: Developmental toxicity study in Rhesus monkeys exposed pre-natally to TCDD through the diet. Altered social interactions were observed in the offspring, LOAEL 5ppt. No guidelines mentioned.

¹² The guidance value is a chronic-duration oral MRL. An uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) was used to derive the MRL.

¹³ Re-evaluation by SCF 2001.

¹⁴ Faqi et al., 1998: Reproductive toxicity study in male offspring Wistar rats exposed to TCDD throughout pregnancy and lactation via subcutaneous injection. Decreased sperm production and altered sexual behaviour in male offspring were observed. No guidelines mentioned.



¹⁵ The TDI of 2 pg/kg/day is rather expressed as a weekly intake (TWI) of 14 pg/kg/day by the committee of SCF and is based on the LOAEL with an uncertainty factor of 9.6.

¹⁶ Ohsako et al., 2001: Developmental toxicity study in Holtzman rats after a single exposure to TCDD on gestational day 15. Ventral prostate weight; decreased anogenital distance in male offspring was observed. No guidelines mentioned.

¹⁷ The TDI of 2.3 pg/kg originates from the PTWI of 70 pg/kg bw/month, which is a midpoint of the range 44-103 pg/kg bw/month. The range of PTWIs is derived from the two studies, Ohsako et al., 2001 and Faqi et al., 1998.

¹⁸ U.S. EPA 2004: draft.

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