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Scientific statement on the health-based guidance values for dioxins and dioxin-like PCBs

European Food Safety Authority

Abstract

Different organisations have undertaken risk assessments of dioxins resulting in the issuance of a range of health-based guidance values. This report examines the approaches taken by the Scientific Committee on Food (SCF), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the United States Environmental Protection Agency (US EPA) and how these differing approaches impact on the final derivation of a numerical value. SCF and JECFA concluded that the critical studies for derivation of a health-based guidance value (HBGV) were animal studies, whereas the US EPA selected the human data, as their preference is to use human data where available. SCF and JECFA applied a body burden one-compartment kinetics approach to derive a HBGV from rat data, whereas US EPA applied physiologically based pharmacokinetic modelling of blood levels estimated from epidemiology studies. An uncertainty factor of 3 was applied by SCF and JECFA as the lowest-observed-adverse-effect level (LOAEL) was close to the no-observed-adverse-effect level (NOAEL) (observed in another animal study), as opposed to the US EPA applying their default uncertainty factor of 10 for extrapolation from a LOAEL in the absence of a NOAEL. This resulted in the reference dose set by US EPA being 3-fold lower than the tolerable weekly intake (TWI)/provisional tolerable monthly intake (PMTI). In view of the different approaches used in the most recent assessments undertaken by the authorities, it would appear appropriate to undertake a comprehensive risk assessment on the risks for animal and human health related to the presence of dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs) in feed and food.

Keywords: dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), dioxin-like polychlorinated biphenyls (dl-PCB), health-based guidance values (HBGVs), tolerable weekly intake (TWI), reference dose (RfD)

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Correspondence: contam@efsa.europa.eu

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1. Introduction

Dioxins are a group of persistent organochlorine compounds that are widely dispersed in the environment and accumulate in the food chain. They are formed as a result of thermal reactions, for example during the incineration of municipal waste and other fires. In addition, dioxins are produced as by-products of various industrial processes such as the bleaching of pulp and paper with chlorine gas and the synthesis of certain chlorinated chemicals like chlorophenols, polychlorinated biphenyls (PCBs) and some chlorophenoxy herbicides. The term 'dioxins' in principle refers to a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, but only 17 of these compounds are thought to be relevant in terms of potential adverse effects for humans and animals. These 17 dioxins contain at least four chlorine atoms with the 2, 3, 7 and 8 positions occupied; in this report the term 'dioxins' refers to these 17 congeners. Due to their resistance to metabolism (and their lipophilic nature), dioxins accumulate in the body. Their biological and toxic effects are similar and mediated by the aryl hydrocarbon receptor (AhR).

PCBs are synthetic organic chemicals. Theoretically, there are 209 possible PCB congeners with 1 to 10 chlorine atoms; of these, the 12 non-ortho or mono-ortho compounds with at least four chlorine atoms exhibit similar biological activity to the PCDDs and PCDFs, so are referred to as 'dioxin-like polychlorinated biphenyls' (dl-PCBs). They are lipophilic chemicals that were widely produced for a range of industrial purposes, e.g. as coolants and insulating fluids, electrical insulators, and in coatings and isolation kits since the 1930's until the 1970's that have entered the environment through both use and disposal. Their use has been discontinued but they may continue to be released during the disposal of materials and obsolete equipment.

Dioxins and dl-PCBs persist in the environment and accumulate in biological systems, as such there continues to be public concern about the health hazards arising from them. For the general population, the primary source of exposure to dioxins and related compounds is food. These compounds are detectable in almost all types of food with the highest concentrations found in meat, fish, eggs and dairy products. However, it is acknowledged that fats and oils contribute a significant proportion of the regular dioxin and dl-PCB intake because they are major components of the diet. Exposure from dietary sources has declined substantially in the last three decades; this is attributed in part to controls upon emissions and the discontinuation of production and use of PCBs.

As the potency of these compounds varies greatly, Toxic Equivalency Factors (TEFs) have been developed to compare the toxicity of the various chemicals and to allow the combined effect of dioxins and dl-PCBs to be assessed. TEFs express the concentrations of other dioxins and dl-PCBs as a concentration equivalent to the most toxic dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), one of the most studied dioxins, for which there are toxicological and epidemiological data available. The weighted concentrations are then summed to give a single value, termed a Toxic Equivalent (TEQ). The TEFs used in the European Union (EU) legislation, by the European Food Safety Authority (EFSA) and by a number of other authorities are those set by the World Health Organization (WHO), as such the resulting TEQs are referred to as WHO-TEQs. TEFs are regularly reviewed and updated based on new information on relative potencies of congeners (last review in 2005).

1.1. Background and Terms of Reference as provided by the requestor

Background

The Scientific Committee on Food (SCF) adopted on 30 May 2001 an opinion on dioxins and dioxin-like PCBs in food,[1] fixing a tolerable weekly intake (TWI) of 14 picogrammes (pg) World Health Organisation toxic equivalent (WHO-TEQ)/kg body weight (b.w.) for dioxins and dioxin-like PCBs.

The Joint Expert Committee on Food Additives (JECFA) of the WHO and from the UN Food and Agriculture Organisation (FAO) established in June 2001 a provisional tolerable monthly intake (PTMI) at 70 pg/kg b.w. for dioxins and dioxin-like PCBs.[2]

[1] Opinion of the Scientific Committee on Food 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://ec.europa.eu/food/fs/sc/scf/out90_en.pdf

[2] WHO Technical Report series, 909. Evaluation of certain food additives and contaminants, p. 121-146. Available at http://whqlibdoc.who.int/trs/WHO_TRS_909.pdf

Converted to a tolerable daily intake, the SCF health-based guidance value of 2 pg/kg b.w. is in line with the JECFA value of 2.3 pg/kg b.w.

The European Food Safety Authority (EFSA) used in 2008 the TWI established by the SCF to estimate the risk for public health due to eth presence of dioxins in pork from Ireland.[3]

In February 2012, the US Environment Protection Agency (US EPA) confirmed the oral reference dose (RfD) of 0.7 pg/kg b.w. per day for dioxins. In addition, the U.S. Agency for Toxic Substances and Disease Registry/Center for Disease Control and Prevention (ATSDR) has established a chronic-duration oral Minimal Risk Level (MRL) of 1.0 pg/kg b.w. per day for dioxins.

It is appropriate that EFSA provides an explanation for the differences in health-based guidance values established by different organizations as regards dioxins and dioxin-like PCBs.

Based on the outcome of the explanation of the differences in these assessments, a comprehensive risk assessment on the risk for animal and public health related to the presence of dioxins and dioxin-like PCBs in feed and food may be needed. If this is the case, the more recent occurrence data of dioxins and dioxin-like PCBs in feed and food need to be taken into account.

Terms of Reference

In accordance with Art. 31 (1) of Regulation (EC) 178/2002, the Commission asked EFSA for **scientific and technical assistance to assess and explain the differences in health-based guidance values established by different organisations as regards dioxins and dl-PCBs**.

Based on the outcome of this scientific statement a comprehensive risk assessment might be needed. If this is the case, the Commission asks EFSA, in accordance with Art. 29 (1) of Regulation (EC) No 178/2002, for a **scientific opinion on the risks for animal and human health related to the presence of dioxins and dl-PCBs in feed and food**, taking into account the recent occurrence data on the presence of dioxins and dl-PCBs in feed and food.

The scientific opinion should, *inter alia*, comprise the:

(a) The evaluation of the toxicity of dioxins and dl-PCBs for animals and humans, considering all relevant adverse acute and chronic health effects;

(b) estimation of the dietary exposure (chronic and, if relevant, acute dietary exposure) of the EU population to dioxins and dl-PCBs including consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following specific diet, etc);

(c) estimation of the exposure of the different animal species to dioxins and dl-PCBs from feed and the levels of transfer/carry-over of dioxins and dl-PCBs from the feed to the products of animal origin for human consumption resulting in unacceptable levels of dioxins and dl-PCBs;

(d) assessment of the chronic (and if relevant acute) human health risks for the EU population including for specific (vulnerable) groups of the population as the consequence of the estimated dietary exposure;

(e) assessment of the animal health risks for the different animal species as the consequence of the estimated exposure from animal feed.

1.2. Interpretation of the Terms of Reference

This Scientific Report of EFSA addresses a request from the European Commission to provide an explanation of the differences in the health-based guidance values established by different authorities, specifically the Scientific Committee on Food (2001), JECFA (2001) and US-EPA (2012).

[3] Available at: http:// http://efsa.europa.eu/en/efsajournal/doc/911.pdf

2. Health-based guidance values established by different authorities

2.1. The Scientific Committee on Food

The Scientific Committee on Food (SCF) last provided advice on risks to human health from exposure to dioxins and dl-PCBs in food to the European Commission in June 2001. The SCF calculated the total amount of TCDD in the fetus (i.e. the fetal body burden) and the associated maternal body burden in the pivotal studies. A specific issue was the difference in the fetal versus the maternal body burden observed during a single or a repeated dosing regimen, the latter being more representative of the average consumer. A factor of 2.6 was derived to correct for this.

Studies of developmental toxicity in rats examining effects upon the reproductive system (Table 1) and the immune system of male offspring were considered by the Committee, effects in studies of the latter being noted to occur at higher doses and as such not considered pivotal. The SCF discussed human studies (in which subtle neurobehavioural effects and changes in circulating hormone levels in newborn infants and children were reported to be associated with their mothers' exposure to dioxin and/or PCB), noting in particular a recent follow-up study on the Seveso cohort by Mocarelli et al. (2000). These studies were considered as not being critical in the derivation of the tolerable intake.

The SCF used both the no-observed-adverse-effect levels (NOAELs) and the lowest-observed-adverse-effect levels (LOAELs) in deriving a tolerable intake for TCDD, using a body burden approach to calculate the equivalent estimated human daily intakes (EHDIs). Results from the single dose studies were corrected because of the differences in fetal body burdens observed between a single and repeated dose, the timing of administration of the dose being critical. Dosing of rats on gestational day 8 (GD 8, i.e. during organogenesis) produces less toxicity than dosing on GD15 for reproductive endpoints. The time of dosing, GD15, at the onset of the endocrine-sensitive phase of sexual differentiation in rats, represents a critical window for fetal exposure for these reproductive endpoints. A dose of 1.0 µg TCDD per kg body weight (b.w.) administered on GD8 produces responses similar to a dose of 0.2 µg TCDD per kg b.w. given on GD15. As fetal concentrations of TCDD measured on GD16 were very similar to those measured on GD15 (Hurst et al., 2000a, b) it was decided that the fetal concentration on GD15 is the critical determinant of these reproductive effects.

In deriving an EHDI, differences in kinetics between rats and humans were taken into account, like the much longer half-life of TCDD in humans. As a result, the SCF concluded that no uncertainty factor was needed to account for toxicokinetic differences between experimental animals and humans. They also concluded that there was no need to apply an uncertainty factor for differences in toxicodynamics between experimental animals and humans and for inter-individual variation among humans, as 'humans are less sensitive to TCDD than responsive rodent strains'. The Committee deemed it appropriate to use the WHO (1994) default uncertainty factor of 3.2 to account for inter-individual variations in toxicokinetics (i.e. absorption, biotransformation, accumulation and elimination of TCDD) in humans, given that the most sensitive effects of TCDD were seen after exposure of female animals, but they had no structured information on the potential variation amongst women regarding the most important determinants in toxicokinetics, i.e. size of body fat stores, CYP1A2 concentrations in liver, and rate of metabolism of TCDD.

Table 1: Estimated steady state body burdens of 2,3,7,8-tetrachlorodibenzo-p-dioxin and associated estimated human daily intakes (EHDI) at NOAEL and LOAELs in the pivotal studies (adapted from SCF, 2001)

Study	Endpoint	NOAEL	LOAEL	Estimated maternal steady state body burden[(a)] (ng/kg b.w.)	Associated EHDI (pg/kg b.w./day)
Mably et al. (1992)	Holzman rats: Decreased sperm count in male offspring		64 ng/kg b.w. single bolus dose by gavage	100[(b)]	50
Gray et al. (1997)	Long Evans rats: Accelerated eye opening and decreased sperm count in male offspring		50 ng/kg b.w. single bolus dose by gavage	80[(b)]	40
Faqi et al. (1998)	Wistar rats: Decreased sperm production and altered sexual behaviour in male offspring		Maintenance of 25 ng/kg b.w. by subcutaneous injections	40[(b)]	20
Ohsako et al. (2001)	Holzman rats: Decreased anogenital distance in male offspring	12.5 ng/kg b.w. single bolus dose by gavage		20[(c)]	10
			50 ng/kg b.w. single bolus dose by gavage	80[(c)]	40

b.w.: body weight; GD: gestational day; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level.
(a): Increment over background. Estimated background body burden from feed in rats is approximately 4 ng TEQ/kg b.w. (assumed to be due to background contamination of the feed).
(b): Composite value resulting from pseudo steady state body burden and acute body burden on GD 15.
(c): Maternal body burden at GD 16.

Applying this uncertainty factor of 3.2 to the EHDI of 10 pg TCDD/kg b.w., calculated from the NOAEL in the Ohsako study, indicated a tolerable intake of 3 pg/kg b.w. per day. When performing the calculation using the LOAELs the Committee found it appropriate to use an additional factor of 3, as the LOAELs were close to the NOAEL, giving an uncertainty factor of 9.6 (i.e. 3 × 3.2). The lowest tolerable intake of 2 pg TCDD/kg b.w. was obtained by applying this uncertainty factor to the EHDI of 20 pg/kg b.w. from the Faqi et al. study (1998). The SCF considered that the Wistar rats used in this study might be the most sensitive rat strain and therefore this value of 2 pg/kg b.w. should be the tolerable intake for TCDD. As TCDD and related compounds have very long half-lives in the human body, the SCF thought it more appropriate to express this as a TWI, extended to include other dioxins and dl-PCBs as a group TWI of 14 pg WHO TEQ/kg b.w.

2.2. The Joint Expert Committee on Food Additives

The Joint Expert Committee on Food Additives (JECFA) of the WHO and Food and Agriculture Organisation (FAO) established a provisional tolerable monthly intake (PTMI) of 70 pg/kg b.w. for dioxins and dl-PCBs in June 2001. Converted to a tolerable daily basis, this equates to a dose of 2.3 pg/kg b.w. per day, comparable with the group TDI of 2.0 pg WHO TEQ/kg b.w. from SCF, and the basis for its derivation is similar.

JECFA calculated the same average maternal and fetal body burdens as those established by SCF after a single dose and repeat doses of TCDD to pregnant Long Evans rats (Hurst et al., 2000a, b). The

Committee assessed the same studies as SCF concluding that a tolerable intake could be established for TCDD on the basis of the assumption that there is a threshold for all effects, including cancer. Thus, they considered the studies summarised in Table 1 to determine the NOAELs and LOAELs, which were provided by the studies of Faqi et al. (1998) and Ohsako et al. (2001), respectively. Following toxicokinetic conversion, these studies give maternal body-burden LOAELs and NOAELs for effects on male offspring of 25 ng/kg b.w. and 13 ng/kg b.w., respectively.

JECFA used the same rationale as the SCF in concluding that an uncertainty factor of 9.6 should be applied to the LOAEL or 3.2 to the NOAEL, resulting in a range of PTMIs from 40–100 pg per kg b.w. per month. The Committee chose 'the mid-point of this range' 70 pg per kg b.w. per month as the PTMI.

2.3. The US Environmental Protection Agency

The US Environmental Protection Agency (US EPA) published an assessment of the non-cancer endpoints for dioxins in February 2012, establishing an oral reference dose (RfD) of 0.7 pg per kg b.w. per day (US EPA, 2012). The US EPA define the oral RfD as 'an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime'. An oral RfD is derived from a benchmark dose lower confidence limit (BMDL), a NOAEL, a LOAEL, or another suitable point of departure (POD) on the dose response relationship, with uncertainty/variability factors applied to reflect limitations of the data used, and as such an oral RfD is comparable to a TDI.

RfDs were derived from epidemiological studies, which the EPA considered first as human data are preferred for derivation of an RfD. The key studies were evaluations of the cohort exposed as a result of the explosion in Seveso, Italy in 1976, i.e. they were directly exposed to TCDD (Alaluusua et al., 2004; Baccarelli et al., 2008; Mocarelli et al., 2008). These studies were deemed appropriate for use in the RfD derivation as exposures were primarily to TCDD, with exposure to other dioxin-like compounds apparently being minimal in addition to exposure associated with background intake. Though these specific publications were not available to SCF and JECFA at the time of their evaluations, these papers constitute repeated follow-up studies on the Seveso cohort, of which publications were available. From each of these studies a POD (shown in Table 2) was estimated from the NOAEL/LOAEL identified for the critical effect observed in the key studies.

Table 2: Points of Departure derived by the US Environmental Protection Agency for epidemiologic studies of TCDD

Study	LOAEL/NOAEL (pg/kg b.w. per day)	Critical effects observed
Alaluusua et al. (2004)	406[(a)] (NOAEL)	Dental effects in adults exposed to TCDD in childhood
Baccarelli et al. (2008)	20[(b)] (LOAEL)	Elevated TSH in neonates; exposure *in utero*
Mocarelli et al. (2008)	20[(c)] (LOAEL)	Decreased sperm count and motility in men exposed to TCDD in childhood

b.w.: body weight; LOAEL: lowest observed adverse effect level; NOAEL: no observed adverse effect level; TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin; TSH: thyroid-stimulating hormone.

(a): Mean of peak exposure (655 pg/kg b.w. per day) and average exposure over 10-year critical window (156 pg/kg b.w. per day).

(b): Maternal exposure corresponding to neonatal TSH concentration exceeding 5 µU/mL.

(c): Mean of peak exposure (32 pg/kg b.w. per day) and average exposure over 10-year critical window (0.80 pg/kg b.w. per day).

The Baccarelli et al. (2008) and Mocarelli et al. (2008) studies were selected to be co-principal studies for the RfD as they were determined to describe the most sensitive endpoints. The endpoints of increased thyroid-stimulating hormone (TSH) in neonates and decreased sperm count and motility were designated as co-critical effects and an adjusted LOAEL of 20 pg/kg b.w. per day was identified as the POD for determination of the RfD. A physiologically-based pharmacokinetic (PBPK) model was

used by the US EPA to simulate the 2,3,7,8-TCDD blood concentrations from these studies, the model and its application are discussed below.

To establish the RfD, EPA applied an uncertainty factor (UF) of 30. The standard factor of 10 (in the absence of information suggesting a lower value) was applied as the RfD was derived from a LOAEL. A factor of 3 was applied to account for human inter-individual variability, i.e. to account for variability from human-to-human because the effects were elicited in sensitive life-stages. The EPA explained that a UF of 1 was not applied because 'the sample sizes in these two epidemiological studies were relatively small, which, combined with uncertainty in exposure estimation, may not fully capture the range of inter-individual variability. In addition, potential chronic effects are not well defined for humans and could possibly be more sensitive'. The resulting calculation gave an RfD for TCDD of 0.7 pg/kg b.w. per day. Although EPA did not overtly extend the RfD to other dioxins, they recommend that the TEF values developed by the WHO should be used for the assessment of mixtures of TCDD and dioxin-like compounds.

2.4. Methodologies

2.4.1. Body burden approach and physiologically-based pharmacokinetic (PBPK) modelling

In their derivation of HBGV, the SCF and JECFA used a body burden approach combined with a simple one-compartment kinetic model based on the assumption of a first-order increase/decrease in the body burden levels as a function of time.

This approach assumes that TCDD is distributed throughout one compartment, i.e. the whole body, without differences in kinetics between specific tissues. Although it is acknowledged that the majority of the TCDD is distributed in lipid stores, at higher doses the liver also selectively sequesters TCDD, due to the binding to cytochrome P450 1A2. However, at the low exposure levels normally experienced by the human population, it was considered appropriate to ignore this liver sequestration and apply simple pharmacokinetics for the transformation of body burdens into estimated human daily intakes. The elimination of TCDD at low doses follows first-order kinetics and is independent of the body burden. The calculation undertaken assumes for humans that 50 % of the TCDD dose from food will be absorbed, and an estimated half-life for TCDD of 2 740 days (7.5 years). For compounds following first order kinetics it will take 3–4 half-lives to approach steady state which for TCDD is equivalent to 20–30 years. This approach is supported by evidence from a simple model (DeVito et al., 1994), which provided reasonably accurate estimates of body burdens in rats that were administered doses of TCDD between 1 and 100 ng/kg b.w. per day in subchronic studies when compared with measurements of actual tissue concentrations.

The US EPA concluded that the assumption of a constant half-life value for the clearance of TCDD from long-term or chronic exposure is not well-supported biologically, given the dose-dependent elimination that is observed in rodents and humans. As such they decided that the dynamic distribution of TCDD between fat, liver and blood as a function of time and dose would be better described using physiologically-based models. These models provide estimates for other dose metrics (e.g. serum, whole blood, or tissue levels) that the US EPA describe as more biologically relevant to response than a body burden estimated based on an assumption of first-order accumulation/elimination over time.

The US EPA considered the potential of different measures as dose metrics of exposure, including first-order body burden, lipid-adjusted serum concentration (LASC), whole blood concentration, tissue concentration, and functional-related metrics of relevance to the mode of action (MOA), for example receptor occupancy. US EPA decided to use the TCDD concentration in whole blood, modelled as a function of administered dose, as the dose metric for assessing TCDD dose response. LASC is often used as the metric for epidemiology studies as TCDD is highly lipid-soluble and LASC accounts for individual differences in the size of the serum lipid compartment. However, US EPA chose to use whole blood concentration because of the structure of the Emond PBPK model, in which the liver and other tissue compartments are connected to the whole blood compartment rather than to the serum compartment. The time-weighted average whole-blood concentration over the relevant exposure periods for all animal bioassay dosing protocols was calculated, by dividing the area under the time-

course concentration curve (AUC) by the exposure duration. In most cases, the reported TCDD LASC was extrapolated both forward and backward in time to simulate the actual exposure scenario.

The epidemiological studies evaluated by the US EPA reported TCDD exposures as LASC, rather than whole-blood concentrations, so oral intakes were modelled using LASC as the dose metric. In the case of the Seveso cohort, there was a single high exposure of TCDD followed by lower level exposures resulting in a gradual decline in the internal TCDD concentrations, so for this cohort both peak and average exposures over a defined period of critical exposure were estimated.

Two biologically based pharmacokinetic models were considered relevant for simulating the TCDD dose metrics in humans and animals exposed via the oral route (Emond et al., 2004, 2005, 2006; Aylward et al., 2005). The US EPA decided that application of the Emond model would be more pertinent than the Aylward model with respect to the scenario being considered, i.e. simulating serum lipid and tissue concentrations during exposures that do not lead to the onset of steady-state condition in the exposed organism. This was because the Emond model is more physiologically-based than the Aylward model, the Emond model simulates the blood compartment directly in the rat, mouse, and human, and the model includes gestational and life-time non-gestational forms, whilst the Aylward model does not. Thus, the choice of the US EPA was to use the Emond rodent PBPK model as the basis to estimate blood TCDD concentrations based on administered doses. Prior to its use, three minor modifications were made to the Emond model: 1. recalculation of the volume of the rest of the body compartment after accounting for volume of the liver and fat compartments; 2. the rate of 2,3,7,8-TCDD excreted via urine was calculated by multiplying the urinary clearance parameter by blood concentration in the equation instead of by the concentration in the rest of the body compartment; 3. the human gastric non-absorption constant was recalibrated to match oral bioavailability data in humans (Poiger and Schlatter, 1986). To assess the effect of these modifications, the 'modified Emond human PBPK model' was then evaluated prior to use by reassessing all published data used in the original model.

2.4.2. Application of the 'modified Emond human PBPK model' to derive an oral reference dose

The dose-response information described in the publications of the three non-cancer epidemiology studies determined whether the US EPA identified a NOAEL or LOAEL to derive a POD. If appropriate information were available, the 'modified Emond human PBPK model' was used to estimate the continuous oral daily intake in ng/kg b.w. per day that would produce the relevant blood TCDD concentrations associated with the NOAEL/LOAEL. If all of this information was available, then the result described in the publication was included as a POD.

All three epidemiological studies considered by US EPA (Table 2) were of the Seveso industrial accident where the cohort was exposed environmentally to high peak concentrations of TCDD. For the dental effects and semen quality endpoints, a POD for derivation of a candidate RfD was calculated by estimating dose as the mean of the peak exposure (following the accident) and the average exposure over a defined critical exposure window for the particular endpoint. For neonatal TSH, the POD was calculated from estimates of maternal exposure during pregnancy as reported in the publication. The POD estimated for tooth development was considerably higher than those estimated for semen quality and neonatal TSH.

2.4.3. Comparison of SCF and JECFA HBGVs with US EPA RfD

The oral RfD of 0.7 pg/kd b.w. per day derived by EPA is approximately 3-fold lower than the HBGVs of approximately 2.0 pg/kg b.w. per day established by SCF (2001) and JECFA (2001) (when expressed on a daily basis). As discussed, the approach for the derivation of the HBGV differs. SCF and JECFA used reproductive toxicity studies in rats rather than the epidemiological data on effects in humans used by US EPA (their preferred basis for derivation of an RfD). SCF and JECFA considered human studies (earlier publications relating details of the repeated follow-up of the Seveso cohort) but they did not consider them as being critical in the derivation of a tolerable intake. Although studies of both rats and humans showed a decreased sperm quality, the effect in rats was observed after *in utero* exposure and in humans after exposure during childhood. As such, a different starting point was used for deriving the HBGV, i.e. mothers of child-bearing age or young boys.

SCF/JECFA applied a body burden/one compartment kinetics approach to derive a HBGV from rat data, whereas US EPA applied PBPK modelling of blood levels estimated from epidemiology studies. The SCF used both the NOAEL (EHDI of 10 pg/kg b.w. per day; Ohsako et al., 2001) and the LOAEL (EHDI of 20 pg/kg b.w. per day; Faqi et al., 1998) from developmental toxicity (considered to be the most sensitive endpoint) studies in rats to derive a tolerable intake for 2,3,7,8-TCDD, using a body burden approach to calculate the equivalent estimated human daily intakes (EHDIs). The US EPA derived two PODs based on the studies of Baccarelli et al. (2008) and Mocarelli et al. (2008), with adjusted LOAELs with the same value of 20 pg/kg b.w. per day. As the studies define the most sensitive endpoints evaluated in the epidemiologic literature, they were designated as co-principal studies for the RfD and were considered to provide mutual quantitative support.

Differences in approach to derive the POD resulted in application of different uncertainty factors to establish the HBGVs. SCF and JECFA applied an uncertainty factor of 9.6 when establishing their HBGV, whereas the US EPA used an uncertainty factor of 30 to establish the RfD. Due to the different origin of the POD (e.g. animal developmental studies selected as pivotal by SCF, and human epidemiology data used by US EPA) there were different uncertainties to be taken into account in the ensuing calculation of a HBGV.

SCF concluded that there was no need to apply an UF for inter-individual variation amongst humans (as it was considered that the most sensitive humans were no more sensitive to TCDD than the responsive rat strains), but considered it appropriate to apply the default UF of 3.2 to account for variability in toxicokinetics between humans, i.e. the inter-individual variations with regard to absorption, biotransformation, accumulation and elimination within the human population.

In contrast, US EPA applied a factor of 3 as the observed effects in humans in the studies they based their calculations upon were elicited at sensitive life stages, applying caution (i.e. not using an UF of 1) as the sample sizes were small and there was uncertainty in estimating the exposure (as described above). In addition, the organisations chose to apply different UFs when basing the calculation on a NOAEL. The SCF thought it appropriate to apply a factor of 3 as the LOAEL was close to the NOAEL (observed in another animal study). In their calculation, the US EPA applied their default UF of 10 for extrapolation from a LOAEL in the absence of a NOAEL, or other information to suggest that it would be appropriate to reduce this factor.

3. Conclusions

- SCF and JECFA concluded that the critical studies for derivation of a HBGV were animal studies whereas the US EPA selected the human data as it is their preferred basis.
- SCF and JECFA applied a body burden one compartment kinetics approach to derive a HBGV from rat data, whereas US EPA applied PBPK modelling of blood levels estimated from epidemiology studies.
- The application of an uncertainty factor of 3 by SCF and JECFA as the LOAEL was close to the NOAEL (observed in another animal study) as opposed to the US EPA applying their default uncertainty factor of 10 for extrapolation from a LOAEL in the absence of a NOAEL results in the RfD being 3-fold lower than the TWI/PMTI.
- In view of the different approaches used in the most recent assessments undertaken by the authorities, it seems appropriate to undertake a comprehensive risk assessment on the risks for animal and human health related to the presence of dioxins and dl-PCBs in feed and food.

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Abbreviations

AhR	Aryl hydrocarbon receptor
AUC	Area Under the Curve
BMDL	Benchmark dose lower confidence limit
b.w.	Body weight
dl-PCBs	Dioxin-like polychlorinated biphenyls
EFSA	European Food Safety Authority
EHDIs	Estimated Human Daily Intakes
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GD	Gestational day
HBGV	Health-based guidance value
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LASC	Lipid-adjusted serum concentration
LOAEL	Lowest-observed-adverse-effect level
MOA	Mode of action
MRL	Minimal Risk Level
NOAEL	No-observed-adverse-effect level
PBPK	Physiologically-based pharmacokinetic
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
PTMI	Provisional Tolerable Monthly Intake
POD	Point of Departure
RfD	Reference dose
SCF	Scientific Committee on Food
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
TSH	Thyroid-stimulating hormone
TWI	Tolerable Weekly Intake
UF	Uncertainty factor
US EPA	United States Environmental Protection Agency
WHO	World Health Organization