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Opinion of the SCF

on the Risk Assessment of

Dioxins and Dioxin-like PCBs

in Food

Adopted on 22 November 2000

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Executive summary

The Committee has been asked to advise the Commission on the scientific basis for the establishment of limits and on any other alternative measures relating to polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) in foods. The PCDDs and PCDFs are collectively referred to as dioxins. In the present opinion, the current situation with respect to the exposure of the general population of European Member States, with particular emphasis on the main sources of dietary exposure, is described and the health significance of these exposures is assessed. In addition, some science-based risk reduction measures have been discussed.

In its deliberations the Committee considered results from recent activities gathering information on the occurrence of dioxins and related compounds in the general environment and in foods, the recent WHO evaluation, the discussions in the CODEX Committee on Food Additives and Contaminants, recent publications and other relevant information.

The Committee focused its opinion on the seventeen 2,3,7,8-substituted PCDDs and PCDFs, and the non-*ortho* and mono-*ortho* PCBs, i.e. the selection of dioxin-like compounds which have been assigned a toxic equivalency factor (TEF) at a WHO Consultation in 1997. This factor is used to calculate the toxic equivalence to 2,3,7,8-TCDD (TEQ). Although they are usually the predominant congeners in environmental samples including foodstuffs, the non-dioxin-like PCBs have been given a lower priority at the present.

The Committee took account of the valuable information on the occurrence of PCDDs, PCDFs and dioxin-like PCBs in food and the dietary exposure to these compounds as compiled in Scientific Cooperation (EU SCOOP) Task 3.2.5. The database provided a collection of national food contamination data and dietary exposure assessments from 10 participating European countries. The Committee examined whether the database provided an adequate basis to estimate the general level of exposure of the population of such countries. To assess the dietary exposure at European level, the national figures contained in the EU SCOOP database were considered and analysed together, which resulted in the following:

- The products of vegetable origin (cereals with less than 2% fat, fruit and vegetables) exhibit very similar dioxin contamination levels, with mean concentrations in the order of 0.02-0.03 pg I-TEQ/g, whole food basis.

- Eggs are characterised by a rather recurrent PCDD and PCDF presence, with a mean around 1 pg I-TEQ/g, lipid basis, and only a slightly higher value of the upper confidence limit.

- Wild fish and farmed freshwater fish exhibit mean contamination levels in the order of 10 pg I-TEQ/g, lipid basis, for dioxins and 30 pg PCB-TEQ/g, lipid basis, for dioxin-like PCBs: the threefold difference of the means appears to reflect a consistently higher contamination from PCBs. The recorded contamination ranges in fish span over two (for dioxin-like PCBs) to three (for dioxins) orders of magnitude.

- On average, poultry, beef and veal, and mixed meat present similar levels of PCDDs and PCDFs (0.5-0.7 pg I-TEQ/g, lipid basis). The estimated mean for pork meat is 0.3 pg I-TEQ/g, lipid basis; however, the difference between this mean and the others is not statistically significant. Game meat and liver present dioxin levels significantly higher than the other meat subgroups. The meat group taken as a whole yields confidence

interval estimates of approximately 0.4-0.7 pg I-TEQ/g for dioxins and 0.3-1.5 pg PCB-TEQ/g for dioxin-like PCBs, both ranges expressed on a lipid basis.

- The subgroup of milk and its products is characterised by somewhat different mean contamination levels, approximately ranging from 0.6 to 1.0 pg I-TEQ/g, lipid basis, for PCDDs and PCDFs, and 0.6 to 1.3 pg PCB-TEQ/g, lipid basis, for dioxin-like PCBs; however, these differences are not significant. The upper confidence limits are in the order of 1 pg I-TEQ/g, lipid basis, for dioxins and fall in the approximate range of 2-10 pg PCB-TEQ/g, lipid basis, for dioxin-like PCBs. Milk contamination figures were obtained by excluding "industrial"-type samples.

In order to give examples for risk management, the frequency distributions of dioxin and dioxin-like PCB contamination levels in a number of foods of animal origin were derived on the basis of the estimated weighted mean and standard deviation figures. The exercise was extended to the determination of the 95th and 99th percentile values, in view of their possible use as cut-off values for acceptance, such as action thresholds, target values and maximum levels. The exercise illustrated that, based on the available distribution curves, cut-off values for acceptance can be readily estimated for any selected percentile value. It should be noticed that a higher percentile level (e.g., the 99th) tends to exclude less of the food material with a higher contamination and consequently saves more of it for the market; therefore, such a choice would be less conservative toward the consumers' protection than that of the 95th percentile. On the other hand, selecting a low percentile, such as the 50th percentile, would have a tremendous impact on both the consumers' exposure and the market.

In the evaluation of the toxicity of dioxins and dioxin-like PCBs, the Committee used as starting point the WHO evaluation of 1998 and expanded the database with studies published since then. Applying the body burden approach, the Committee arrived at a temporary tolerable weekly intake (t-TWI) of 7 pg/kg bw for 2,3,7,8-TCDD. For 2,3,7,8-TCDD and related compounds, such as other dioxins and dioxin-like PCBs that have very long half-lives in the human body, the Committee found it more appropriate to establish a temporary tolerable weekly intake (t-TWI) instead of a tolerable daily intake (TDI).

The Committee concluded that the t-TWI for 2,3,7,8-TCDD could be extended to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs, and established a group t-TWI of 7 pg WHO TEQ/kg bw for these compounds.

Using the current database of dietary exposures to dioxins and dioxin-like PCBs it can be concluded that a considerable proportion of the European population will exceed the group t-TWI of 7 pg WHO-TEQ/kg bw. The Committee wishes to emphasise that a TWI is not a lower bound of toxicity, it is an estimate of a safe level of intake and is derived conservatively using uncertainty factors applied to no observed adverse effect levels (NOAELs) or lowest observed adverse effect levels (LOAELs). However, that does not necessarily mean that there is an appreciable risk to the health of individuals exceeding slightly the group t-TWI, but exposure above this tolerable weekly intake leads to an erosion of the protection embedded in the group t-TWI.

Various risk assessments of PCDDs, PCDFs and dioxin-like PCBs have identified groups of the population that may experience higher than average exposure through high consumption of heavily contaminated food, human milk (breast-fed infants), or occupational exposure. It is important to note that the sensitive endpoints of the studies that drive the derivation of the t-TWI relate to the body burden of dioxin in fertile women. The intake by breast-fed infants

was mimicked in the studies that were considered during the derivation of the t-TWI, in which offspring were exposed through the suckling phase. In this context, the Committee reiterated the conclusions of the WHO meetings on the health significance of contamination of human milk with dioxins and PCBs, namely that the current evidence does not justify altering recommendations on the promotion of, and support for, breast-feeding.

The Committee considered the extension of the WHO-TEF approach to other compounds, such as the brominated dioxin analogues, but concluded that the database for these compounds was inadequate to carry out an assessment. It was aware of a suggestion that hexachlorobenzene should be included in the WHO-TEF scheme but considered that the data did not justify such an inclusion.

It should also be noted that the dietary exposure to dioxins in some European countries has decreased since the end of the 1980s by approximately 50%. Nevertheless, because intake estimates indicate that the t-TWI is being regularly exceeded by a considerable proportion of the European population, there is still a need for risk reduction strategies. Therefore, continuous efforts should be made to limit then environmental release of PCDDs, PCDFs and dioxin-related compounds.

The Committee discussed some risk management strategies as to how reduction in dietary exposure to dioxins and dioxin-like PCBs could be achieved. The Committee stresses that continuing efforts should be made to limit release of PCDDs, PCDFs and dioxin-related compounds to the lowest levels that are technically achievable. It is the opinion of the Committee that this is the most efficient way to reduce the presence of these compounds in the food chain and to ensure continued reductions in human body burdens. Additional strategies may include setting of maximum levels, action thresholds, and target values using information on current levels in foods.

Terms of reference

The Committee is asked to advise the Commission on the scientific basis for the establishment of limits and on any other alternative measures relating to polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) in food. The Committee is asked to give particular attention to:

- the general level of exposure of the EU population relative to reported toxicological limits;
- the identification of the main sources of dietary exposure and the relative importance of dietary and non dietary sources;
- the identification of high risk sub-groups of the population;
- the identification of gaps in knowledge, which limit the Committee's assessment.

In its deliberations the Committee is asked to consider the results of recent activities gathering information on the occurrence of dioxins and PCBs in foods, the WHO evaluation, the discussions in the CODEX Committee on food additives and contaminants, and any other relevant information.

In addition to the above assessment, the Committee is invited to give consideration to possible science-based risk reduction measures.

Background to the terms of references

The health significance of human exposure to dioxins and PCBs has been subject of extensive discussions. The main source of human exposure is food (Fürst *et al*., 1992).

The most recent assessment of the risks for human health from dioxins and PCBs was performed in 1998[1], when a WHO Consultation agreed on a tolerable daily intake (TDI) of PCDD/PCDFs and dioxin-like PCBs in the range of 1-4 pg Toxic Equivalents (TEQ)/kg body weight (bw). The Consultation recommended that new Toxic Equivalency Factors (TEFs) for PCDD/PCDFs and dioxin-like PCBs should be used for future calculations of TEQs. The experts also recognised that subtle effects may already occur in the general population in developed countries at the current background dietary exposure levels of 2-6 pg TEQ/kg bw/day.

In its opinion of 16 June 1999[2] the SCF took account of the results of the WHO Consultation. The Committee concluded that the Tolerable Daily Intake value as set by the WHO group is, in general terms, adequate to provide the basis for risk management in this area. However, it wished to revisit the WHO evaluation, giving special attention to whether a weekly rather than a daily tolerable intake should be considered given the cumulative nature of the substances and the extrapolation from the LOAELs for the most sensitive endpoints in animal

[1] Executive summary, Assessment of health risk of dioxins: Re-evaluation of the Tolerable Daily Intake (TDI), WHO Consultation, May 25-29 1998, http://www.who.int/pcs/pubs/dioxin-exec-sum/exe-sum-final.html (last time accessed 23 November 2000). See reference WHO (2000).

[2] Opinion of the Scientific Committee on Food on Dioxins in milk derived from cattle fed on contaminated feed in Belgium, expressed on 16 June 1999.

experiments, *via* body burden comparison between animals and humans, and the use of an uncertainty factor of 10, used by WHO to derive the TDI range of 1-4 pg TEQ/kg bw.

In view of an assessment of the risk for consumer health from dioxins and PCBs in food products the SCF also suggested that more information on background levels of dioxins in food and its incidental sources should become available.

The report of a compilation exercise on dioxin exposure data (AEA Technology, 1999) concludes that further analysis of major contributors to dietary exposure in Member States is required, as well as identification of risk groups within the EU population. Consumption of fish from certain sources can add significantly to the total exposure to dioxins and PCBs (Svensson *et al*., 1991). Therefore, measures related to fish consumption have already been taken by some Member States (Hallikainen and Vartiainen, 1997).

Additional information on dietary exposure to dioxins is expected from the Scientific Cooperation (SCOOP) Task 3.2.5 carried out under Council Directive 93/5/ECC.

Another conclusion of the report by AEA Technology cited above points out that "...many citizens of EU Member States may have a daily intake of PCDD/PCDFs and PCBs in excess of the WHO recommended TDI." The report goes on to say that "maximum tolerable concentrations of PCDD/PCDFs and PCBs should be established for key foodstuffs in Europe, with a view to setting limit or guideline values to be met by food producers." Also the SCF in its recent opinion stated that "The Committee is of the opinion that it would be possible to establish permanent limits for dioxins and PCBs in foods that take into account long-term exposures of the population".

In fact, the recent contamination crisis of the Belgian food supply highlighted the absence of such harmonised limits. This absence greatly increases the difficulty of management of such problems at Community level.

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1. Introduction

The term "dioxins" encompasses a group of 75 polychlorinated dibenzo-*p*-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners. Although dioxins are not produced by intention except for research and analytical purposes, these contaminants have an ubiquitous distribution due to their formation as unwanted and often unavoidable by-products in a number of anthropogenic activities. PCDDs and PCDFs are formed during incomplete combustion processes, industrial as well as natural. They occur also as contaminants during various industrial processes, e.g. the chemical manufacture of some chlorinated compounds and chlorine bleaching of paper pulp.

The toxicity of individual dioxin and dibenzofuran congeners differs considerably. The congeners that are of toxicological importance are substituted in each of the 2-, 3-, 7- and 8-positions. Thus, from 210 theoretically possible congeners, only 17 are of toxicological concern. These compounds have a similar toxicological profile to that of the most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). The toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, reproductive and developmental toxicity and most, if not all, are mediated *via* the aryl hydrocarbon (Ah) receptor present in most tissues of animals and humans.

Polychlorinated biphenyls (PCBs) are chlorinated aromatic hydrocarbons which are synthesised by direct chlorination of biphenyl. Depending on the number of chlorine atom substituents (1-10) and their position on the two rings there are 209 theoretically possible congeners. Due to their physical and chemical properties, such as non-flammability, chemical stability, high boiling point, low heat conductivity and high dielectric constants, technical PCB mixtures were widely used in a number of industrial and commercial open and closed applications. Closed applications have included the use of PCBs in hydraulic and heat transfer systems as well as cooling and insulating fluids in transformers and capacitors. Typical open applications have been the use of PCBs in pigments, dyes, repellents and carbonless copy paper or as plasticizers in paints, sealings, plastics and rubber products. It is estimated that more than one million tonnes of technical PCB mixtures have been produced and marketed world-wide since their first commercial use in the late 1920s. The production and use of PCBs has been discontinued in most countries, but large amounts remain in electrical equipment, plastic products, buildings and the environment.

PCBs have been divided into three groups according to their biochemical and toxicological properties. Non-*ortho* and mono-*ortho* substituted PCBs show toxicological properties that are similar to dioxins, and are potent inducers of the cytochromes CYP1A1 and CYP1A2, which are markers of Ah receptor-mediated biochemical and toxicological effects. They are therefore often termed "dioxin-like PCBs". Most other PCBs do not show dioxin-like toxicity and many are inducers of CYP2B1 and CYP2B2 in the liver of rodents. Among the non-*ortho*, mono-*ortho* and di-*ortho*-substituted PCBs some are "mixed-type" inducers, increasing both CYP1A and CYP2B enzyme activities. However, the ability of the di-*ortho* substituted PCBs to produce Ah receptor-mediated dioxin-like toxicity is negligible.

Due to their comparable toxicological properties, it was considered appropriate to include the dioxin-like PCBs, i.e. non-*ortho* and mono-*ortho* PCBs, in the risk assessment and risk management of the dioxins. In contrast, because of their lower toxicity, non-dioxin-like (di-*ortho*) PCBs, which are normally the predominant congeners in environmental samples, including foodstuffs, have been assigned a lower priority at the present.

Dioxins and PCBs are lipophilic compounds. They are extremely resistant towards chemical and biological degradation processes and therefore persist in the environment and accumulate in food chains.

1.1. Toxic equivalency factors and previous assessments of PCDDs, PCDFs and dioxin-like PCBs

Two different approaches have been used world-wide in the risk assessments of PCDDs, PCDFs and dioxin-like PCBs. WHO and most countries outside the USA have derived Tolerable Daily (or weekly) intakes (TDI) for 2,3,7,8-TCDD in the range of 1-10 pg/kg bw/day, assuming the existence of a threshold dose for the carcinogenic effect of TCDD (see Annex III, Table 1). In contrast, in the United States the Environmental Protection Agency (EPA) has used probabilistic estimates of cancer potency, treating cancer as a non-threshold effect, in the derivation of a Risk Specific Dose (RsD) as low as 0.006 pg/kg bw/day (US EPA, 1989, 1994). In the current EPA reassessment, inclusion of recent data has resulted in an upper bound cancer risk at background exposure levels for the general population that is ten fold higher than the estimate in its previous assessment (Farland *et al.*, 2000).

In almost all matrices, dioxins and PCBs are not found as single compounds but as complex mixtures. In order to facilitate the comparison of analytical and exposure data, it has proved useful to convert the analytical results into toxic equivalents (TEQ). This conversion is based on the assumption that all 2,3,7,8-substituted PCDDs and PCDFs, as well as the dioxin-like PCBs, bind to the same receptor, the Ah receptor, and show comparable qualitative effects, but with different potencies. These differences in toxicity are expressed in the toxic equivalency factors (TEFs), estimated from the weaker toxicity of the respective congener in relation to the most toxic congener 2,3,7,8-TCDD, which is assigned the arbitrary TEF of 1. By multiplying the analytically determined amounts of each congener by the corresponding TEF and summing the contribution from each congener the total TEQ value of a sample can be obtained using the following equation:

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

Several different TEF schemes have been proposed. Until recently the most widely used schemes have been the International TEFs (I-TEFs) (NATO/CCMS, 1988) for PCDDs and PCDFs and the WHO-ECEH (European Centre for Environment and Health of the World Health Organization) scheme for PCBs (PCB-TEFs, Ahlborg *et al.*, 1994) (Table 1). In June 1997, WHO-ECEH and the International Programme on Chemical Safety (IPCS) arranged an international meeting in Stockholm which resulted in a consensus on the TEFs for PCDDs, PCDFs and dioxin-like PCBs for both human (WHO-TEFs, Table 1) and fish and wildlife risk assessment (van den Berg *et al.*, 1998). Depending on the model used, a conversion of the same analytical raw data into TEQ values may differ due to different TEFs for certain congeners (Table 1). These differences have to be taken into account when results calculated with different TEF models are compared. In food and human samples, dioxin TEQ values based on WHO-TEFs are approximately 10-20% higher than those obtained by using the I-TEFs of NATO/CCMS.

TABLE 1. Comparison of most widely used TEFs for dioxins and dioxin-like PCBs.

PCDDs and PCDFs	**Toxic Equivalency Factor (TEF)**	
	I-TEF (NATO/CCMS, 1988)	WHO-TEF (van den Berg *et al.*, 1998)
2,3,7,8-TCDD	1	1
1,2,3,7,8-PnCDD	0.5	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.001	0.0001
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PnCDF	0.05	0.05
2,3,4,7,8-PnCDF	0.5	0.5
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.001	0.0001

PCBs (IUPAC number)	**Toxic Equivalency Factor (TEF)**	
	PCB-TEF (Ahlborg *et al.*, 1994)	WHO-TEF (van den Berg *et al.*, 1998)
Non-*ortho PCBs*		
3,3',4,4'-TCB (77)	0.0005	0.0001
3,4,4',5-TCB (81)	-	0.0001
3,3',4,4',5-PnCB (126)	0.1	0.1
3,3',4,4',5,5'-HxCB (169)	0.01	0.01
Mono-*ortho PCBs*		
2,3,3',4,4'-PnCB (105)	0.0001	0.0001
2,3,4,4',5-PnCB (114)	0.0005	0.0005
2,3',4,4',5-PnCB (118)	0.0001	0.0001
2,3,4,4'5-PnCB (123)	0.0001	0.0001
2,3,3',4,4',5-HxCB (156)	0.0005	0.0005
2,3,3',4,4',5'-HxCB (157)	0.0005	0.0005
2,3',4,4',5,5'-HxCB (167)	0.00001	0.00001
2,3,3',4,4',5,5'-HpCB (189)	0.0001	0.0001
Di-*ortho PCBs*		
2,2',3,3',4,4',5-HpCB (170)	0.0001	-
2,2',3,4,4',5,5'-HpCB (180)	0.00001	-

Abbreviations: 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.

TEFs for dioxin-like compounds apply only to Ah receptor-mediated responses. The criteria used by WHO for including a compound in the TEF scheme for dioxin-like compounds were that the compound must:

- show a structural relationship to the PCDDs and PCDFs,
- bind to the Ah receptor,
- elicit Ah receptor-mediated biochemical and toxic responses, and
- be persistent and accumulate in the food chain.

To determine TEFs for mammals the WHO-ECEH/IPCS Consultation in 1997 followed a tiered approach in which the results of animal toxicity studies, especially those involving (sub)chronic exposure, were given significantly more weight than the results of *in vitro* or biochemical studies (van den Berg *et al.*, 1998).

The use of this concept assumes dose additivity. While additivity predominates in the majority of experimental studies, non-additive interactions of PCDD, PCDF and PCB mixtures, in particular antagonistic effects, have been reported at greater than environmental levels of exposure. These non-additive effects are considered to be due to multiple mechanisms of action of individual congeners and/or to pharmacokinetic interactions. For the mono-*ortho* PCBs especially, certain endpoints such as carcinogenicity, porphyrin accumulation, alterations in circulating thyroid hormone concentrations and neurotoxicity could arise by both Ah receptor-mediated and non-Ah receptor-mediated mechanisms. This increases the uncertainty in the use of TEFs for mono-*ortho* PCBs. In addition, non-Ah receptor-mediated mechanisms of action may be shared by certain di-, tri-, and tetrachloro *ortho*-substituted PCBs normally also present in the environment and food (van den Berg *et al.*, 1998).

The WHO Consultation in 1998 stressed that the TEF is an order of magnitude estimate of the toxicity of a compound relative to 2,3,7,8-TCDD, using careful scientific judgement after considering all available data (WHO, 2000).

Annex I of the Opinion presents the statistical assessment of PCDD, PCDF, and dioxin-like PCB levels in European foodstuffs, Annex II presents the summary of health effects of dioxins, and Annex III provides the background, concepts and studies considered in the risk assessment of dioxins and dioxin-like PCBs in food.

2. Exposure assessment

2.1. Sources of human exposure

Humans are exposed to dioxins and/or PCBs through either:

- accidental exposure,
- occupational exposure, or
- environmental exposure.

In the last few decades, several accidents have caused extensive exposure of humans to dioxins and dioxin-related compounds. Well-known examples are the exposure of the local population at Seveso (Pocchiari *et al.*, 1979; Bertazzi and di Domenico, 1994), and from fires in PCB-filled electrical equipment, such as in the Binghamton State Office Building (Fitzgerald *et al.*, 1986, 1989). High exposures, with toxicity, may be caused by the ingestion of accidentally contaminated food items. Known examples are the contamination of edible oils, such as the Yusho (Japan) and Yu-Cheng (Taiwan) food poisoning episodes (Rogan *et al.*, 1988; Kuratsune *et al.*, 1996).

Occupational activities in which TCDD and related compounds are unintentionally produced, such as waste incineration or production of certain pesticides or chemicals, may also result in a significant human exposure.

While accidental and occupational dioxin exposure is normally limited to more or less small subgroups of the population, environmental exposure due to diffuse sources affects all humans. This exposure is possible by several routes:

- food consumption,
- inhalation of air and ingestion of particles from air,
- ingestion of contaminated soil,
- dermal absorption.

While the last three routes normally contribute less than 10% to the total daily dioxin intake, more than 90% of human dioxin exposure derives from food. Of this, about 90% normally comes from foods of animal origin (Fürst *et al.*, 1992). Contamination of food is primarily caused by release of dioxins from various sources (e.g., waste incineration, production of chemicals, metal industry), and their subsequent accumulation in the food chain where they are particularly associated with fat. Other sources may include contaminated feed, as occurred in Belgium in 1999 (Ashraf, 1999; Broeckaert and Bernard, 2000), application of sewage sludge to farm land, flooding of pastures, waste effluents, and certain types of food processing and preparation.

2.2. Occurrence in food and dietary exposure

Human exposure to dioxins and related compounds has been the subject of various international studies conducted in the past few years. For instance, a very informative document containing an extensive compilation of data on concentrations of PCDDs and PCDFs in food and human milk, and the resulting dietary exposure of the general population, is Volume 69 of IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

(IARC, 1997). In October 1999, AEA Technology presented a comprehensive report in the framework of a project entitled "Compilation of EU Dioxin Exposure and Health Data" (AEA Technology, 1999); this report includes data until 1995.

2.2.1. The EU SCOOP database

On June 7, 2000, the final report of Scientific Cooperation (EU SCOOP) Task 3.2.5, entitled "Assessment of Dietary Intake of Dioxins and Related PCBs by the Population of EU Member States", was released. This project was carried out at the request of the European Commission and jointly coordinated by The Netherlands and Sweden. The objective of this specific task was to provide the European Commission with information on dietary exposure to PCDDs, PCDFs, and dioxin-like PCBs in the participating countries. Ten countries (Belgium, Denmark, Finland, France, Germany, Italy, The Netherlands, Norway, Sweden, and the United Kingdom, hereafter referred to as "participating countries") delivered the available data on the occurrence of PCDDs, PCDFs, and dioxin-like PCBs in food products and human milk. Samples were obtained at national levels, from various sites, including rural and industrial sites, and collected in different years covering the period 1982-1999.

Wherever available or possible, data on consumption of these foods and the dietary exposure of the general population to these compounds were provided. Along with the data, relevant supporting information was collected on their quality together with an evaluation of whether the data were representative of the country that had released them, and therefore relevant for use in estimating national dietary intakes. This judgement was provided by each participating country, for its own data.

The EU SCOOP database compiles information already published by IARC (IARC, 1997) and AEA Technology (1999), but it also contains more recent material resulting from studies conducted until the end of 1999. In addition, it contains information on dioxin-like PCBs that was not taken into consideration in the other reports.

The Committee noted that there were large differences in the amount, detail, and quality of the data from the participating countries. In particular, analytical results appear to have been obtained largely without adequate harmonisation of analytical procedures and/or intercalibrating processes of the laboratories in the different countries, this ultimately influencing comparability of the results. In addition, some data may be slightly overestimated due to a lack of sufficient sensitivity of the determination in the analytical laboratories that performed the assays. In these cases the "upperbound approach" was applied in the EU SCOOP report, i.e. the limit of determination was used in calculating TEQs for those congeners that were not quantitated.

2.2.1.1. Main results of the EU SCOOP Task 3.2.5

The EU SCOOP Task 3.2.5 resulted in a comprehensive database with information on concentrations of PCDDs, PCDFs, and/or dioxin-like PCBs in food products and human milk. The most recent data (1995-1999), relevant to the present opinion, can be summarised as follows (for the meaning of I-TEQs and PCB-TEQs, see Section 1.1).

- The available information on the occurrence of dioxins in foods consumed in the participating countries shows national average concentrations of PCDDs and PCDFs in eggs, fats and oils, meat (products), and milk (products) in the order of 1 pg I-TEQ per

gram (g) of extracted lipids, or less. PCDD and PCDF levels in fish are on average in the order of 10 pg I-TEQ/g, lipid basis. In comparison, concentrations in fruits, vegetables, and cereals were found to be relatively low, and were generally close to the limits of determination. The limited information with respect to concentrations of dioxin-like PCBs indicates average TEQ contributions of one to three times the TEQ contribution of PCDDs and PCDFs.

- The average dietary exposure of PCDDs and PCDFs for an adult person has been estimated to be between 0.4 and 1.5 pg I-TEQ/kg bw/day. For surveys based on chemical analyses of foods collected in the 1970s and 1980s, intakes were estimated to be higher, ranging from 1.7 to 5.2 pg I-TEQ/kg bw/day. Based on data from the United Kingdom and The Netherlands, the 95th-percentile intake in general appears to be two to three times the mean intake.

- The average dietary exposure to dioxin-like PCBs appears to be between 0.8 and 1.5 pg PCB-TEQ/kg bw/day. In studies investigating dietary exposures of both dioxins and PCBs, the TEQ contribution of dioxin-like PCBs was estimated to be from almost equal (e.g., Finland, The Netherlands, Sweden, the United Kingdom) to approximately four times (Norway) the TEQ contribution of PCDDs and PCDFs.

- The main contributors to the average daily dietary exposure to dioxins (I-TEQs) appear to be milk and dairy products (16-39%), meat and meat products (6-32%), and fish and fish products[1] (11-63%). Other products, mainly of plant origin such as vegetables, cereals, and fruit, contributed some 6-26% in those countries for which data were available.

- As to the above point, it should be noted that the ranking of the food groups in their contribution to the total intake of I-TEQs differed from country to country. These differences may result from different food consumption habits in the participating countries, but other factors may also be involved. These include the sampling strategy applied (e.g., differences in the coverage of products collected to represent a whole food group), and the large variations in concentrations of dioxin-related substances in some of the food groups. It has already been noted that some data may be overestimated. This would apply primarily to samples of vegetable nature.

- In most countries, young children have a higher dietary exposure to dioxins and dioxin-like PCBs per kg bw than adults.

- On a body weight basis, the intake of breast-fed infants has been estimated to be one to two orders of magnitude higher than the average adult intake.

- A considerable amount of data exists for concentrations of PCDDs and PCDFs in human milk. For the period before 1995, the national average levels ranged from 10 to 34 pg I-TEQ/g, lipid basis. For the period 1995-1999, the national average concentrations appear to range from 7.9 to 19 pg I-TEQ/g, lipid basis, for some countries clearly indicating a downward trend.

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[1] It has to be noted that throughout this Opinion, the terms “fish and fish products” are used. These terms have to be understood in the context of this Opinion, in line with the SCOOP report, to include fish, fish products, crustaceans and molluscs.

- The database is not sufficiently complete to draw a firm conclusion about the TEQ contribution of dioxin-like PCBs in human milk. The results of a few studies carried out in the period 1990-1994 indicate mean PCB-TEQ concentrations comparable to the I-TEQ contributions of PCDDs and PCDFs over the same time period.

- A few countries (i.e., Finland, Germany, The Netherlands, Sweden, and the United Kingdom) reported sufficient data for the establishment of time trends in the dietary exposure. These data reveal clearly that the exposure of the general population to dioxins has declined since the late 1980s. For Germany, Finland, The Netherlands, and Sweden this decline is also noted for concentrations in human milk.

2.2.2. Patterns of the distribution of contamination in selected European foods

2.2.2.1. Dietary exposure assessment

The assessment of exposure to a contaminant through the diet ideally requires accurate and specific data for a reliable characterisation of both food contamination and food consumption distributions. If these data are available, the assessment is based on two successive steps:

- the determination of the main food categories that contribute most to human exposure (for lipophilic contaminants such as PCDDs, PCDFs, and PCBs, they normally coincide with foods of animal origin);
- the examination of the contaminant distribution in each of the selected food categories.

The EU SCOOP database provides a collection of national food contamination data and exposure assessments from 10 participating countries. The Committee examined whether the database provided an adequate basis on which to estimate the general level of exposure of the population of such countries. For the purpose of assessing the exposure at European level, the aforesaid national figures were considered and analysed together.

As to the first step of the work (i.e., determination of main food contributors to dioxin and dioxin-like PCB intake), a reliable characterisation of both food contamination and food consumption distributions was obtained from the EU SCOOP database. Dioxin and dioxin-like PCB contamination levels in foods of animal origin are generally expressed on a lipid basis. Consequently, reliable fat content figures for the priority food categories identified are needed in order to carry out an exposure assessment. However, such figures cannot be derived from the EU SCOOP database as they were either not explicitly reported or they originated from analytical programmes possibly not designed for exposure assessment. One way to circumvent this shortcoming is to derive fat content figures from food composition tables. However, due to the heterogeneity of the tables available at the EU level, the use of those figures would increase the uncertainty of the assessment, when compared to the national exposure assessments already available from the EU SCOOP report. Therefore, as the Committee could not derive a reliable estimate of the average European exposure, it decided to use national data to determine which food categories give a significant contribution to human exposure (see Section 2.2.1.1).

For the second step (i.e., the analysis of a contaminant distribution in selected food categories at European level), the national results presented in the EU SCOOP database could be used as a starting point. However, the Committee recognised the limitations of the database and the difficulties in comparing average contamination levels per food category, as reported by the participating countries, as these figures were based on data sets of varying size. To provide an

assessment of the distribution of contamination in priority food categories at European level, the Committee made a number of assumptions for the selection and combination of data. A description of the statistical procedure adopted and the results derived are reported in the following subsections and, more extensively, in Annex I.

2.2.2.2. Analysis of the raw data in the EU SCOOP report

The EU SCOOP database contains a large and unique set of PCDD, PCDF, and dioxin-like PCB concentrations in many foods of the participating countries. For the reasons outlined in Section 2.2.2.1, the set of these concentrations was subjected to statistical analysis in order to obtain a group of descriptors (means, standard deviations, confidence limits, etc.) suitable to describe the distributions of the specific food data making up the data set itself (see Annex I, "Statistical Assessment of PCDD, PCDF, and Dioxin-like PCB Levels in European Foodstuffs"). Only data since 1995 were selected for the evaluation as, owing to the downward trend of environmental levels of the toxicants, the use of older data did not seem to be appropriate for a characterisation of current food contamination.

In the EU SCOOP report, most concentration data appear to have been released by the countries after pooling of samples at the origin or averaging of analytical results, so that the number of data (N1) is actually less than the number of physical samples (N2) they represent. For instance, for a few observations on eggs (N1 = 13), a much larger number of physical samples (N2 = 1300) were originally collected. Where deemed appropriate, the N2 values were used to obtain weighted estimates of means and standard deviations. The way original findings were released to fill in the EU SCOOP database is expected to have limited the spreads of data distributions (see Section 2.2.2.3).

Clustered data concerning a primary food group were eventually divided into subsets associated with more homogeneous food subgroups. This was done in order to yield a better insight into the statistical features of the contamination and more elements for intragroup comparison. Subgrouping concerns in particular *Fish and fish products*, *Meat and meat products*, and *Milk and milk products*. A synopsis of selected outcomes from Annex I is shown in Table 2. The statistical descriptors presented were obtained through a log-transformation passage as, in most cases, the normality of log-transformed data appeared to be improved when compared to that of the original distributions (generally tailed towards high values).

The estimated means (<X>) and 99% confidence intervals (CIs) summarised in Table 2 may be considered the "best estimates" of the average contamination in the foods dealt with; the ranges of the original average concentration data come from the EU SCOOP database. It may be noticed that, in some instances, the CI width exceeds the matching data range. This is explained by the combination of two factors: a small value of N1, the parameter used to determine the degrees of freedom for the CI estimate, and the expected limited spreads of the distributions of original data, as already mentioned. In summary, the following may be observed (concentration values are rounded).

- According to the CIs, the products of vegetable origin (cereals with less than 2% fat, fruit, and vegetables) exhibit very similar dioxin contamination levels, with mean concentrations in the order of 0.02-0.03 pg I-TEQ/g, whole food basis.

Table 2. Synopsis of PCDD, PCDF and dioxin-like PCB concentrations in European foodstuffs since 1995. The estimated means (<X>) and confidence intervals (CIs) come from the statistical evaluation of the EU SCOOP database, June 2000 (see Annex I). Concentrations are expressed in pg TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg TEQ/g, whole food basis).[a]

FOOD GROUP	PCDDs + PCDFs			DIOXIN-LIKE PCBs[b]		
	<X>	CI(99%)[c]	RANGE[d]	<X>	CI(99%)[c]	RANGE[d]
Cereals and cereal products[e]	0.019[f]	0.004-0.081	0.010-0.020	0.110[g]	—	—
Eggs	1.19	0.895-1.57	0.460-7.32	—	—	0.440-1.45[f]
Fish and fish products	9.80	6.57-14.6	0.125-225	30.7	17.5-53.8	1.61-168
—Wild fish (marine, freshwater)[i]	9.92	6.34-16.2	0.125-225	35.3	16.7-74.9	1.61-168
—Freshwater fish (culture)	8.84	5.54-14.1	2.33-27.9	19.6	9.03-42.4	9.92-39.7
Fruit and vegetables	0.029	0.014-0.063	0.004-0.090	—	—	0.030-0.120[g]
Meat and meat products	0.525	0.387-0.712	0.130-3.80	0.674[f]	0.303-1.50	0.090-3.15
—Poultry	0.524	0.355-0.774	0.370-1.40	—	—	0.590-0.700[f]
—Beef and veal	0.681	0.499-0.929	0.380-1.10	0.914[f]	0.342-2.44	0.860-1.08
—Pork	0.258	0.174-0.381	0.130-3.80	—	—	0.090-0.810[f]
—Game	1.81[f]	0.403-8.15	0.970-1.97	3.15[g]	—	—
—Others: liver	2.27	1.12-4.59	0.950-3.29	—	—	0.270-1.65[g]
mixed meat	0.540[f]	0.043-6.76	0.270-0.760	0.430[g]	—	—
Milk and milk products[h]	0.882	0.720-1.08	0.260-3.57	1.07	0.411-2.79	0.230-1.80
—Milk as such[h]	0.972	0.749-1.26	0.260-3.57	1.25	0.115-13.6	0.230-1.80
—Others	0.612	0.555-0.675	0.300-1.50	0.564[f]	0.181-1.76	0.380-0.780

[a] TEQ contributions estimated through the I-TEF system for PCDDs and PCDFs and the PCB-TEF system for PCBs (Ahlborg *et al.*, 1994).
[b] Where CIs are absent, original data were insufficient for statistics.
[c] Two-tail probability estimates. For an explanation why a CI may be larger than the matching data range, see text.
[d] Original data from the EU SCOOP database.
[e] Fat content <2%.
[f] Contributions from two participating countries.
[g] Contribution from one participating country.
[h] Data associated with "industrial"-type specimens omitted.
[i] Also includes some farmed salmon.

- Eggs are characterised by a rather recurrent PCDD and PCDF presence, with a mean around 1 pg I-TEQ/g, lipid basis, and only a slightly higher value of the upper confidence limit.
- Wild fish and freshwater fish from farms exhibit indistinguishable contamination levels, on average centered around 10 pg I-TEQ/g, lipid basis, for dioxins and 30 pg PCB-TEQ/g, lipid basis, for dioxin-like PCBs: the threefold difference of the means appears to reflect a consistently higher contamination from PCBs, also confirmed by the corresponding upper confidence limits. In spite of the small spreads exhibited by the CIs, the recorded contamination ranges in fish span over two (for dioxin-like PCBs) to three (for dioxins) orders of magnitude.
- Poultry, beef and veal, and mixed meat have similar concentrations of PCDDs and PCDFs. The estimated <X> and CIs for pork meat are approximately half of those reported for the other three subgroups; however, this difference is not statistically significant. Game meat and liver have concentrations of PCDDs and PCDFs significantly higher than the other meat subgroups. If the *Meat and meat products* group is conservatively taken as a whole, this yields CI estimates for PCDDs and PCDFs and dioxin-like PCBs spanning, respectively, the approximate intervals of 0.4-0.7 pg I-TEQ/g and 0.3-1.5 pg PCB-TEQ/g, both on the lipid basis.
- Lastly, the subgroups of milk ("milk as such") and its products ("others") are characterised by mean contamination levels within a factor of 2 for both groups of analytes (approximately ranging from 0.6 to 1.0 pg I-TEQ/g or 0.6 to 1.3 pg PCB-TEQ/g, lipid basis); however, differences are not significant. The upper confidence limits are in the order of 1 pg I-TEQ/g, lipid basis, for PCDDs and PCDFs and fall in the approximate range of 2-10 pg PCB-TEQ/g, lipid basis, for dioxin-like PCBs. The contamination values reported for milk were obtained by excluding data associated with "industrial"-type samples.

2.2.2.3. Patterns of distribution of PCDD, PCDF and dioxin-like PCB contamination and their possible use

In order to provide examples for risk management, the frequency distributions of contamination levels were derived for a number of foods on the basis of the means (μ) and standard deviations (σ) estimated for the log-transformed PCDD, PCDF and dioxin-like PCB data (see Annex I).

It may be recalled that concentrations in individual samples provide the best data for the construction of distribution curves, but this information is in general not available at European level. However, the distribution curves derived in this opinion are considered to be the best available representation of the contamination situation as they were obtained from the best estimates of means and standard deviations of a very large amount of food contamination data.

With reference to Figures 1-3, these distributions are discussed hereafter; additional details are available in Annex I. The distribution patterns of PCDDs and PCDFs in eggs and milk (and its products) appear to cover ranges of reduced spread. It may be observed that both eggs and milk come exclusively from farming, this involving a relatively limited number of species and feeding habits generally controlled by humans. On the contrary, as was already noticed, dioxin and dioxin-like PCB distribution patterns in fish (and fish products) spread over two to three orders of magnitude. This is readily explained by the large variety of organisms taken into account, including farmed as well as wild fish, fish from various regions, and fish

belonging to different levels of the trophic web up to high level predators, the latter often showing the effects of very pronounced biomagnification processes. For meat (and meat products), mostly produced through farming and exhibiting an intermediate data spread, the values on the right end of the distribution are associated with a few unusually high dioxin levels in pork meat (Figure 2) and with minor contributors such as liver and game. However, both fish and meat are characterised by severely skewed distributions of dioxin and dioxin-like PCB concentrations (Figures 1 and 3), reflecting the remarkable dispersion of the contamination values found in the two food groups.

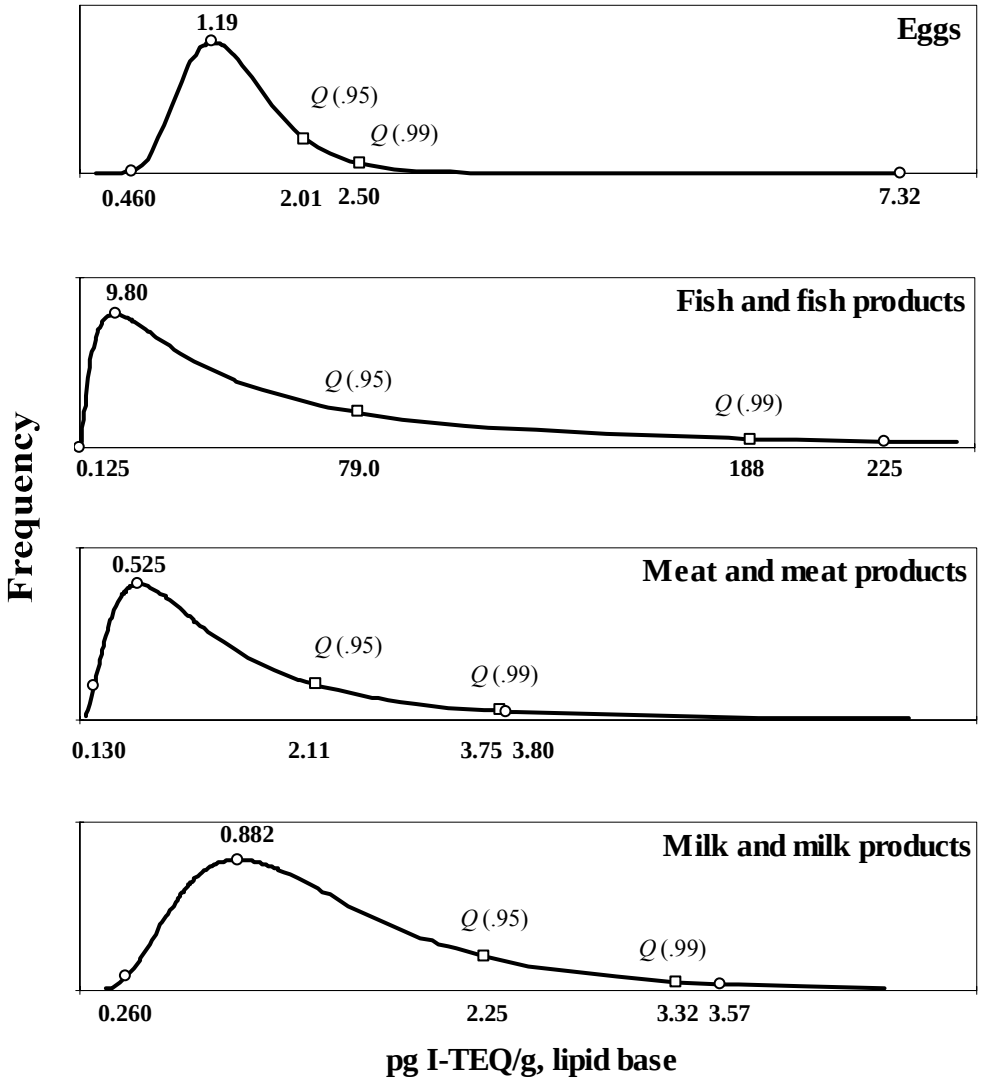

Figure 1. Frequency distribution curves of PCDD and PCDF concentrations in various foods of animal origin. The concentration figures identified by (o) are, from left to right, X_{min}, <X>, and X_{max}. The 95th and 99th percentiles are identified from left to right by (□). The range covered by the fish distribution is between one and two orders of magnitude greater than those of the other foods.

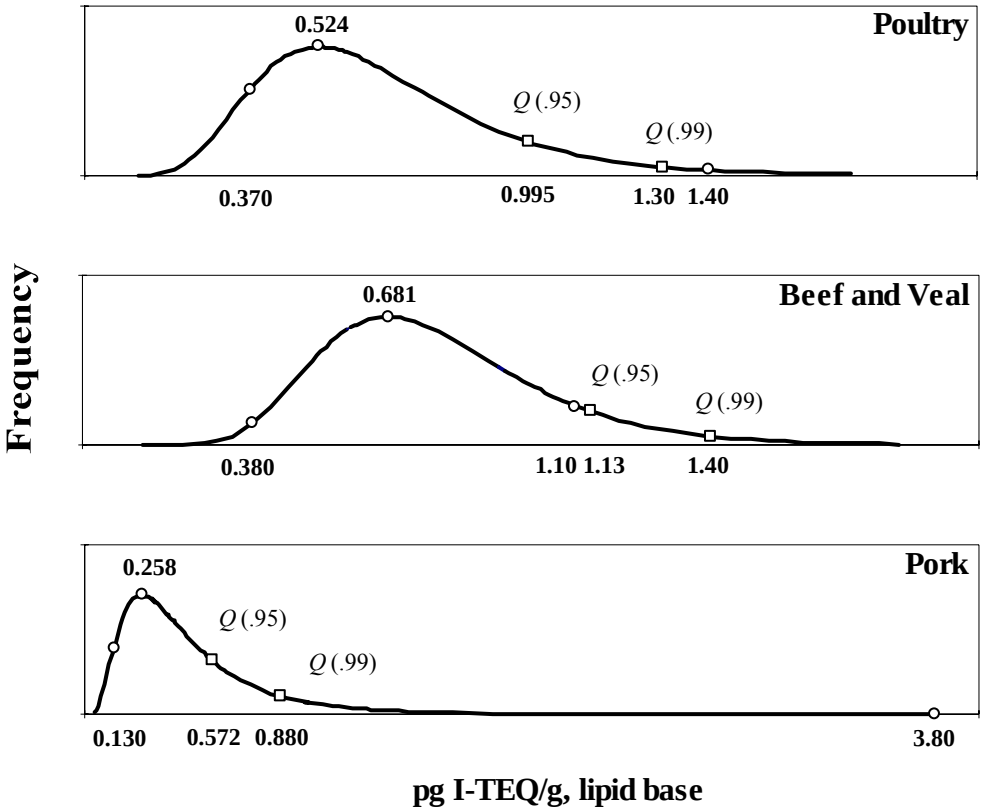

Figure 2. Frequency distribution curves of PCDD and PCDF concentrations in three food subgroups of animal origin. The concentration figures identified by (o) are, from left to right, X_{min}, <X>, and X_{max}. The 95th and 99th percentiles are identified from left to right by (□).

In Figures 1-3, five numerical values have been superimposed on the frequency distribution curve of each food category. From left to right, they are: X_{min}, <X>, the 95th and 99th percentiles (Q(.95) and Q(.99)), and X_{max}. For PCDDs and PCDFs in beef and veal (Figure 2) and dioxin-like PCBs in fish and meat (Figure 3), one or both percentile estimates exceed X_{max}. This may be explained, as mentioned before, by the fact that the frequency distribution patterns are based on data sets essentially containing "means" (more than one sample → one result, from either averaging single results or pooling samples at the origin) rather than individual results (one sample → one result). In other words, potential extreme concentration values at both ends of the distributions would be expected to be absent as they are already included in the means presented by the EU SCOOP database.

The estimated 95th and 99th percentile values shown in the figures are only intended to provide examples of how to use the statistical descriptors (namely, the aforementioned μ and σ) to derive the frequency distribution patterns of contaminants in different foods and therefrom identify eventual cut-off points for risk management. It is recalled here that a concentration level associated with a given percentile (e.g., the 95th) divides the data into two subsets: 95% of the data will fall below that level, whereas the remaining 5% will be higher.

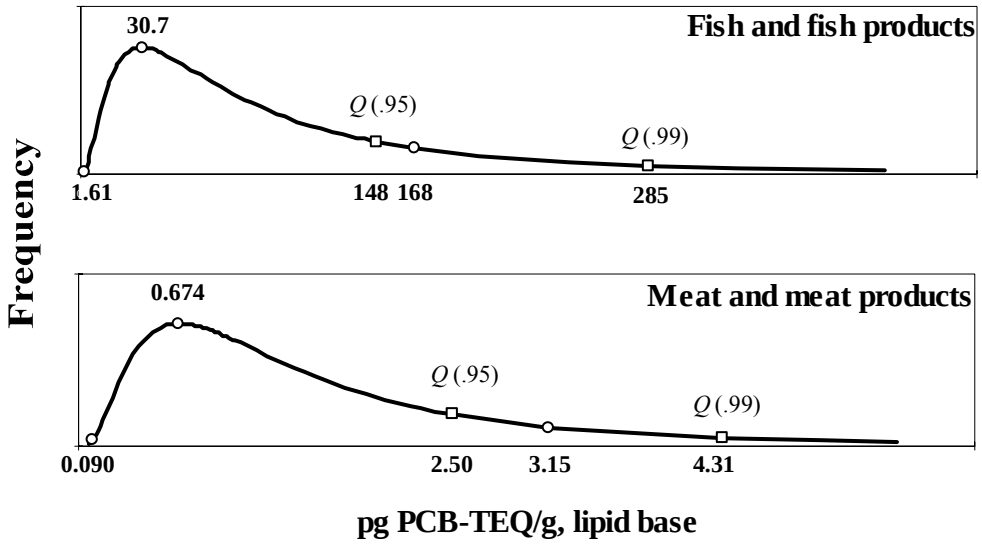

Figure 3. Frequency distribution curves of dioxin-like PCB concentrations in foods of animal origin. The concentration figures identified by (o) are, from left to right, X_{min}, <X>, and X_{max}. The 95th and 99th percentiles are identified from left to right by (□). The range covered by the fish distribution is between one and two orders of magnitude greater than that of the meat food group.

Therefore, using the frequency distribution patterns of dioxins in Figure 1, a cut-off for acceptance set, for example, at 2 pg I-TEQ/g, lipid basis, would keep on the market 95% of the eggs, almost 95% of the milk and its products, almost 95% of the meat, but would keep most of the fish and fish products out of the market. Again as an example, the value for keeping approximately 95% of the fish and fish products on the market would need a cut-off for acceptance of around 80 pg I-TEQ/g, lipid basis. As a higher percentile level (e.g., the 99th) tends to exclude less of the food material with a higher contamination and therefore save more of it for the market, this type of choice should be regarded as less conservative toward the consumers' health than that of the 95th percentile. Selecting a low percentile (e.g., the 50th) would have a tremendous impact on both consumers' exposure and market. As a last observation, the magnitudes of the *Q*(.95) and *Q*(.99) values for a given frequency distribution may vary considerably relative to one another (e.g., for eggs the *Q*(.99) estimate is only some 25% higher than the value associated with *Q*(.95), whereas for fish the difference is over 100%).

From the frequency distribution patterns for poultry and beef and veal of Figure 2, the 95th percentiles appear to be well below the cut-off value for acceptance of 2 pg I-TEQ/g, lipid basis, chosen above as an example. In fact, the *Q*(.95) estimates are in the order of 1 pg I-TEQ/g, lipid basis. For pork meat, a cut-off value for acceptance of 1 pg I-TEQ/g, lipid basis, is even above the 99th percentile, the *Q*(.95) estimate being around 0.6 pg I-TEQ/g, lipid basis. The long tail to the right of the frequency distribution pattern for pork meat is essentially due to a few samples with particularly high contamination levels.

A similar approach could be applied to the dioxin-like PCBs; however, the limited database available did not allow more than two frequency distribution curves to be determined (Figure 3).

The examples given above illustrate that, based on the available distribution curves, cut-off values for acceptance can be readily estimated for any selected percentile value.

It may be pointed out that the cut-off values for acceptance given above were expressed on the extractable lipid ("fat") amount. However, the level of these lipids may vary remarkably even within the same food category (e.g. milk, fish). In particular, concentrations of chemicals such as dioxins and PCBs in fish depend on their fat content, the extent to which wild fish migrate, the number of times they spawn, and their age, site, and feeding habits (Larrson *et al*., 1996). If this is taken into account, it would probably be appropriate to define risk management measures, based on risk assessment figures, by using cut-off values for acceptance expressed on whole food basis.

3. Toxicological evaluation

3.1. Introduction

The Committee focused its considerations on the recent re-evaluation of dioxins and dioxin-like PCBs carried out by a WHO Consultation in 1998. At that meeting a tolerable daily intake (TDI) for humans as a range of 1-4 pg WHO-TEQ/kg bw was recommended for dioxins and dioxin-like PCBs. The Consultation also stressed that the upper range of the TDI of 4 pg TEQ/kg bw should be considered a maximal tolerable intake on a provisional basis and that the ultimate goal is to reduce human intake levels below 1 pg TEQ/kg bw/day. Until the summer of the year 2000 only an executive summary of the WHO evaluation was available to the public (WHO, 1998). However, the Committee had access to some of the draft background monographs that formed the basis for the WHO evaluation (abstracted in Annex II). The full report of the WHO Consultation is now available (WHO, 2000).

Key points in the WHO evaluation were:

- body burdens were used as dose metrics rather than daily doses in order to compare across species;
- the evaluation deviated from most earlier evaluations of 2,3,7,8-TCDD by not using the liver toxicity or the carcinogenicity reported in long-term rat studies as critical endpoints for derivation of the TDI;
- although studies in humans were carefully evaluated, the evaluation was ultimately based on sensitive endpoints in experimental animals;
- developmental, reproductive and hormonal effects following 2,3,7,8-TCDD exposure of female rats and monkeys were identified as the most sensitive adverse effects reported, i.e. the adverse effects occurring at the lowest body burdens;
- the use of the most updated TEF scheme for dioxins and dioxin-like PCBs, i.e. WHO TEFs (van den Berg *et al.*, 1998) was advocated for calculation of the total TEQ in mixtures of these compounds;
- based on the evaluation of 2,3,7,8-TCDD, the WHO Consultation expressed the TDI as 1-4 pg WHO-TEQ/kg bw.

The Committee discussed the principles used in the WHO assessment and performed an evaluation of the studies that were used in the establishment of the WHO TDI. Relevant information that had become available from studies published since the WHO Consultation in 1998 was also examined.

More extensive descriptions of the studies described here can be found in Annex III.

3.2. Use of body burden as dose metric

Many of the biochemical and toxicological effects following dioxin exposure are the same irrespective of whether the intake is acute or chronic. The lethal effect of acute dioxin exposure is largely independent of dose, as long as it exceeds a lethal dose (i.e. animals do not die faster because the dose is 10 or 100 times a lethal dose). For effects such as enzyme

induction, immunotoxicity, developmental toxicity and a number of other toxic endpoints the responses are directly associated with tissue concentrations and not with the daily dose. The key determinants of the kinetics and the half-lives of these compounds are the size of and concentration in the fat stores in the body, the binding to CYP1A2 in the liver and the rate of metabolism and excretion. Differences in these determinants, in particular in the rate of metabolism and excretion, result in large differences in 2,3,7,8-TCDD half-lives across species, ranging from, for instance, 20 to 30 days in rats, over approximately 400 days in monkeys, and between 5 and 11 years in humans. Thus, 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. From a pharmacokinetic point of view, body burden estimations are therefore considered a more appropriate dose metric for interspecies comparisons than the daily dose.

The most appropriate dose metric would ideally be the concentration at the target tissue. However, it is seldom known. DeVito and colleagues (1995) found that for a number of effects that have clearly been associated with dioxins, humans and animals respond at similar body burdens. Therefore the body burden of 2,3,7,8-TCDD in the experimental animals of a study with a sensitive endpoint is the most appropriate surrogate to use 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 can be 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:

Body Burden at steady state (ng/kg bw) = f * Intake (ng/kg bw/day) * half-life in days/ln(2)

where f is the fraction of dose absorbed (assumed to be 50% for absorption from food for humans), and an estimated half-life for 2,3,7,8-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.

This model assumes that dioxins are distributed in only one compartment (the whole body). Although the majority of the dioxin body burden is distributed in the lipid stores of the body, it has been found that in animals and humans, at higher doses, the liver also sequesters these compounds to some extent. However, at the low exposure levels experienced by the general human population, the Committee considered that the application of simple classical pharmacokinetics is appropriate for the transformation of body burdens into estimated human daily intakes. In support, DeVito *et al.* (1995) reported that such a simple model provided reasonably accurate estimates of body burdens in rats that were administered between 1 and 100 ng 2,3,7,8-TCDD/kg bw per day in subchronic studies when compared with estimates that were based on measurements of actual tissue concentrations.

In the following, the Committee has estimated the 2,3,7,8-TCDD body burdens of the experimental animals in the studies providing the most sensitive LOAELs for 2,3,7,8-TCDD. From these estimated body burdens the Committee calculated the associated estimated human daily intakes (EHDI) using the equation given above.

3.3. Effects considered as not being critical in the derivation of the tolerable intake

A number of effects produced by dioxins in experimental animals and observed in occupationally or accidentally exposed humans are clearly high dose effects. Many of the non-cancer effects observed in adult male workers occupationally exposed to high levels of 2,3,7,8-TCDD and higher chlorinated dioxins were transient effects disappearing after the end of exposure. A few conditions appear to be persistent and in excess amongst highly exposed cohorts when compared to unexposed referent groups. These include alterations in lipids, elevated fasting plasma glucose and γ-glutamyl-transpeptidase concentrations, as well as mortality from cardiovascular disease. An association between high dioxin exposure and diabetes mellitus has been reported in some studies whereas other studies were not able to support this association.

A number of biochemical changes, such as enzyme induction, altered expression of growth factors and enhanced oxidative stress have been noted in experimental animals at 2,3,7,8-TCDD body burdens within a lower range of 3-10 ng/kg bw. The Committee considered these biochemical effects to be early markers of exposure to dioxins or events induced by dioxin-like compounds in animals and in humans that may or may not result in adverse effects at higher body burdens.

The Committee also noted a very recent follow-up study of the Seveso cohort which indicated that increased paternal body burden of 2,3,7,8-TCDD might be associated with a decreased sex ratio of the offspring (Mocarelli *et al.*, 2000). Paternal serum lipid concentrations of 2,3,7,8-TCDD higher than 118 pg/g lipid at the time of conception were associated with the birth of significantly more girls than boys. A serum concentration of 15 pg 2,3,7,8-TCDD/g lipid was considered a background baseline level. The use of multivariate models provided results which indicate that this decreased M/F sex ratio might already be apparent at serum 2,3,7,8-TCDD concentrations between 15 and 80 pg/g lipid in the father, corresponding to body burdens of 3 and 16 ng 2,3,7,8-TCDD/kg bw assuming a body fat content of 20%. It would require daily intakes of 1.5 or 8 pg 2,3,7,8-TCDD/kg bw, respectively, for 20-30 years to approach these body burdens.

The derivation of a tolerable intake useful for protection of the general population should be based on the most sensitive adverse effects observed in either humans or experimental animals after dioxin exposure. However, the Committee considered that a discussion of the carcinogenic properties of dioxins was necessary, although this effect does not appear to be the most important adverse effect in relation to background dioxin exposure. In addition, the Committee 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 mother's exposure to dioxin and/or PCBs.

3.3.1. Carcinogenicity

The International Agency for Research on Cancer (IARC, 1997) has classified 2,3,7,8-TCDD as a human carcinogen (Group 1) based on limited evidence in humans and sufficient evidence in experimental animals as well as on mechanistic considerations.

The overall evaluations of other dioxins were:

- "other polychlorinated dibenzo-*p*-dioxins are not classifiable as to their carcinogenicity to humans (Group 3)",
- "dibenzo-*p*-dioxin [unsubstituted] is not classifiable as to its carcinogenicity to humans (Group 3)",
- "polychlorinated dibenzofurans are not classifiable as to their carcinogenicity to humans (Group 3)".

The Committee considered updated epidemiological studies and the available experimental data and, based on these and on mechanistic information, concluded that 2,3,7,8-TCDD produces tumours at multiple sites in experimental animals and, from a qualitative point of view, should be regarded as a human carcinogen.

The Committee also concluded that 2,3,7,8-TCDD is not a direct-acting genotoxic agent. It has not shown effects in the majority of assays for genotoxicity and it does not bind covalently to DNA. 2,3,7,8-TCDD does increase oxidative stress, resulting indirectly in DNA damage. However, the relevance of this to the carcinogenicity of 2,3,7,8-TCDD has yet to be established. Several studies have shown that 2,3,7,8-TCDD is a tumour-promoting agent in experimental animals, in particular in studies of liver tumour promotion.

Several modes of action for the tumour promotive effect of 2,3,7,8-TCDD have been hypothesised:

- binding to the Ah receptor mediates an increase in the expression of genes involved in cell growth and differentiation,
- induction of cytochrome P450 (CYP1A1 and CYP1A2 expression) mediates oxidative stress which leads to increased DNA-damage,
- inhibition of apoptosis favours expansion of pre-neoplastic cell populations.

Based on the available information, the application of a threshold model was considered by the Committee to be appropriate for the indirect and non-genotoxic action of 2,3,7,8-TCDD.

In the long-term carcinogenicity study in rats by Kociba and coworkers (1978) in which an increase in liver tumours was observed, the LOAEL (10 ng/kg bw/day) corresponds to a steady state body burden of 294 ng 2,3,7,8-TCDD/kg bw. In order for humans to obtain a similar steady state body burden a daily intake of 150 pg/kg bw is needed. Body burdens of 109-7000 ng/kg bw (retrospectively estimated from blood concentrations of 2,3,7,8-TCDD in occupational cohorts in which there was limited evidence of a human cancer response) overlap with the estimated body burden of 2976 ng 2,3,7,8-TCDD/kg bw in the highest dose group (100 ng/kg bw/day) of the Kociba study (WHO, 2000), in which a clear carcinogenic effect was observed. This indicates that the rat, based on body burden comparison, is more sensitive than humans to the carcinogenic potency of 2,3,7,8-TCDD, but within less than one order of magnitude.

The Committee noted that the 2,3,7,8-TCDD body burdens in female rats showing an increased incidence in liver tumours and the 2,3,7,8-TCDD body burdens associated with an increased cancer risk in the human cohorts occupationally or accidentally exposed, were

several orders of magnitude higher than the background dioxin body burdens seen in the general population.

3.3.2. Effects in children following pre- and postnatal exposure

Multiple, persistent effects were observed among children whose mothers had been exposed during pregnancy to high levels of PCBs and PCDFs as a result of 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 (Rogan *et al*., 1988; Kuratsune *et al*., 1996).

Neurodevelopmental delays and neurobehavioural effects, including neonatal hypotonia, have been reported in three cohorts, two in the USA followed since 1980 (Gladen *et al*., 1988; Gladen and Rogan, 1991; Jacobson and Jacobson, 1996) and one in The Netherlands followed since 1990 (Koopman *et al*., 1994; Koopman *et al*., 1996; Huisman *et al*., 1995). In the two cohorts from the USA the findings were related to high exposure to "total"-PCB in human milk as measured by a crude method. Dioxins and dioxin-like PCBs were not measured. In the study in The Netherlands, *in utero* exposure to dioxins and dioxin-like PCBs may have influenced early neurodevelopmental parameters and thyroid hormone status, e.g. of thyroxine (T4) and thyroid stimulating hormone (TSH), in infants up to 3 months of age. In this study, maternal body burdens were estimated from maternal milk. Transient differences in these parameters were reported when the high exposure group (above 63 ng total TEQ/kg fat in human milk) was compared with the low exposure group (below 63 ng total TEQ/kg fat in human milk). A concentration of 63 ng TEQ/kg fat in human milk would correspond to a maternal body burden of 12.6 ng/kg bw, assuming that the body contains 20% fat. It should be noted that the observed differences in neurodevelopmental parameters and hormone levels in these environmentally exposed cohorts were subtle, within the normal range and considered without clinical relevance. The associations observed were considered to be due to prenatal (*in utero*) exposure rather than to postnatal exposure (to human milk). Breast-fed infants performed better in neurobehavioural tests than bottle-fed infants (Koopman *et al*., 1996).

The interpretation of these results is complicated by the simultaneous exposure to non-dioxin like PCBs and potentially a large number of other persistent compounds. However, these subtle effects have been demonstrated at body burdens of dioxins and dioxin-like PCBs only slightly higher than that of the average general population. In addition, it should be recognised that the ubiquitous presence of these compounds in humans may have complicated the identification of a normal state with respect to these parameters.

More details are given in Annex II.

3.4. Sensitive effects in experimental animals considered in the derivation of the tolerable intake

The Committee focused its evaluation on adverse effects reported in studies in which 2,3,7,8-TCDD was administered at low doses. The most sensitive adverse effects reported are developmental and reproductive effects in rats and monkeys and an increase in the incidence of endometriosis in monkeys. For most of these effects, NOAELs were not identified in the studies available, only LOAELs. The Committee describes and comments on these studies in Sections 3.4.1-3.4.4. The WHO Consultation used these LOAELs to estimate the body

burdens in the experimental animals and calculated the associated estimated daily human intakes. The Committee noted that the estimations of the 2,3,7,8-TCDD body burdens of the animals in these studies were not documented in the WHO executive summary (WHO, 1998). The Committee discusses these body burdens in Section 3.5.

3.4.1. Effects on the learning behaviour in the offspring of 2,3,7,8-TCDD-exposed rhesus monkeys

The potential effects of perinatal 2,3,7,8-TCDD exposure on learning behaviour in the offspring of rhesus monkeys fed 2,3,7,8-TCDD-containing diet have been reported by Schantz and Bowman (1989). Groups of eight female rhesus monkeys, 6-10 years old, were administered 2,3,7,8-TCDD in the diet at concentrations of 0, 5, or 25 ng/kg diet for up to four years. Birth cohorts were delivered at mean times of 16.2 and 36.3 months. While the high dose group was allowed to mate they delivered too few viable offspring and were therefore not included in the study. The exposure of the offspring ceased at weaning at 4 months. Ten exposed and ten control monkeys were subjected to tests for cognitive recognition, namely four discrimination reversal learning (DRL) tests (spatial, spatial discrimination with irrelevant cues, colour and shape, i.e. object learning), commencing at the age of 14 months, and a delayed spatial alternation (DSA) test at 20 months. There were no significant group mean differences between control and 2,3,7,8-TCDD-exposed animals with respect to acquisition measures taken in all tests and the reversals in the spatial, spatial discrimination, and color DRL tests, as well as the DSA test. However, the group of 2,3,7,8-TCDD-exposed animals was significantly retarded in trials to criterion on the first reversal after acquisition (but not on the seven subsequent reversals) for the shape DRL test. This performance on the shape DRL test was negatively correlated with 2,3,7,8-TCDD body fat concentration as measured at 5 months of age.

Comments

It is noteworthy that only one significant 2,3,7,8-TCDD-related, possibly deleterious, difference between the groups was reported, namely a deficit in one test of object learning. However, the 2,3,7,8-TCDD exposed offspring were only significantly retarded on the first reversal of the shape DRL test though not impaired on acquisition of this test and they were not retarded on the seven subsequent reversals. In this test and that of colour learning there was a negative correlation to 2,3,7,8-TCDD body fat concentrations at 5 months of age, i.e. improvements. Although the authors claim that the sensitivity of the DRL model is well established and that impaired performance on the DRL occurs in the absence of any impairment on the DSA task, the Committee was of the opinion that this subtle, non-persistent change is of doubtful significance for humans. In addition, the Committee noted that the offspring of either birth cohort were combined into one group and that there was an inconsistent association of possible adverse effects with 2,3,7,8-TCDD body fat concentration.

Conclusion

In one study, chronic dietary exposure of female rhesus monkeys at 5 ng 2,3,7,8-TCDD/kg of diet 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 (Schantz & Bowman, 1989). The Committee was of the opinion that this subtle, non-persistent change is of doubtful significance for humans.

3.4.2. Effects of 2,3,7,8-TCDD exposure on the development of endometriosis in rhesus monkeys

The female rhesus monkeys administered 0, 5, or 25 ng of 2,3,7,8-TCDD per kg of diet (8 animals per group) for up to four years (3.5 years in the 5 ng/kg diet group and 4 years in the 25 ng/kg diet group) and whose offspring had been used in the study of learning behaviour were observed by Rier and colleagues (1993) for a further 10 years following cessation of 2,3,7,8-TCDD exposure. During the observation period, three animals in the high dose group died as a result of severe endometriosis. At the end of the observation period the surviving monkeys were diagnosed for endometriosis by laparoscopy. Endometriosis was diagnosed in 33% of the controls, 71% of the low dose group and 86% of the high dose group. The increased incidence in the high dose group was statistically significant. Moderate to severe endometriosis (stages II, III and IV according to human criteria) was not seen in control animals, but was diagnosed in 43% of the low dose group and 71% of the high dose group. Linear regression analysis of the severity of the disease was correlated with the cumulative 2,3,7,8-TCDD dose administered. The authors reported that the prevalence of spontaneous endometriosis in monkeys housed in the colony was 30%.

Comments

Endometriosis occurs exclusively in menstruating species, e.g. humans and non-human primates. In monkeys the disease models human disease anatomically and clinically, though it is reported to be potentially fatal in monkeys. The onset of endometriosis in the animals occurred some years after exposure ended. The results for incidence and severity of endometriosis in the groups fed 2,3,7,8-TCDD were statistically significant and related to the cumulative 2,3,7,8-TCDD dose.

In support of this observation, growth of surgically-induced endometriotic cysts has been promoted in rats and mice following exposure to 2,3,7,8-TCDD (and 2,3,4,7,8-PnCDF), although at much higher doses than in the monkey. Inclusion of prenatal exposure to 2,3,7,8-TCDD at GD8 increased the size of endometriotic lesions promoted by adult exposure in mice, but not in rats. Preliminary studies in two human cohorts have reported increased body burdens of dioxin and dioxin-like PCBs in women suffering from endometriosis.

Conclusion

Female rhesus monkeys fed a diet containing 5 or 25 ng 2,3,7,8-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 2,3,7,8-TCDD exposure. The severity of the disease was correlated with the cumulative 2,3,7,8-TCDD exposure and the increased incidence in the high dose group was statistically significant (Rier *et al.*, 1993). Thus, chronic administration of 5 ng 2,3,7,8-TCDD per kg of diet represents a sensitive LOAEL for endometriosis in the female rhesus monkey.

3.4.3. Effects on the reproductive organs in the offspring of 2,3,7,8-TCDD-exposed rats

The first studies to report on the effects of prenatal 2,3,7,8-TCDD exposure on reproductive development in the male offspring of rats were published in 1992 in a series of three papers (Mably *et al.*, 1992a, b, c). Pregnant Holzman rats were treated by gavage with single 2,3,7,8-TCDD doses of 0.064, 0.16, 0.4, or 1 μg/kg bw on gestational day 15 (GD15), which marks the onset of sexual differentiation in the rat. The male offspring were examined at several

time-points up to 120 days of age. A maternal 2,3,7,8-TCDD dose as low as 0.16 μg/kg bw altered the androgen status, resulted in a decrease in the anogenital distance at birth, delay in testicular descent, decrease in seminal vesicle and ventral prostate weights, and changes in masculine sexual behaviour. The lowest maternal dose tested (0.064 μg 2,3,7,8-TCDD/kg bw) produced a decrease in epididymis and cauda epididymis weights, daily sperm production, and cauda epididymal sperm number. No significant effect on fertility was seen.

In studies performed by Gray and colleagues (Gray and Ostby, 1995; Gray *et al.*, 1995, 1997a, 1997b) pregnant Long Evans Hooded rats were administered 0.05, 0.20, 0.80, or 1.0 μg 2,3,7,8-TCDD/kg bw by gavage on GD15 and the male and female offspring were examined up to 15 months of age. Although there was no indication of altered androgen status in these animals, most of the effects previously seen in the male offspring of Holzman rats were confirmed. The percentage of male pups displaying eye opening at postnatal days 14 and 15 was significantly increased in all treatment groups and at 15 months ejaculated sperm counts were reduced in all dose groups. However, the reduction in sperm count (25%) in the low dose group was not significantly different from that of the control group. In the female offspring a maternal dose of 0.2 μg 2,3,7,8-TCDD/kg bw or higher produced external malformations of the genitalia. The absence of changes in serum oestradiol concentrations and ovarian oestradiol synthesis indicated that the malformations were not a consequence of abnormal ovarian function during prepubertal development.

Comments

The bioavailability of 2,3,7,8-TCDD to the foetus at a given maternal body burden may differ between a bolus dose (as in these rat studies) and dietary exposure at steady state. Thus, it has been shown that gavage dosing of rats and mice with corn oil solutions of benzyl acetate resulted in approximately 200-fold greater peak plasma concentrations of the metabolite benzoic acid than daily administration of the equivalent total dose of benzyl acetate in the diet (Yuan *et al.*, 1995). Intuitively, differences in foetal bioavailability would therefore seem likely. Given that placental transfer will be mediated *via* the blood, it is serum rather than tissue levels that will be critical in determining the magnitude of foetal exposure. Following a bolus administration, serum 2,3,7,8-TCDD levels would be elevated before redistribution to the tissue compartments. In contrast, low-level chronic exposure will not significantly elevate serum levels. The time of dosing, GD15, marks the onset of the endocrine-sensitive phase of sexual differentiation in rats and therefore represents a critical window for foetal exposure for these reproductive endpoints. Thus, studies have shown that dosing of rats on GD8 (i.e. during organogenesis) produced less toxicity than dosing on GD15 for these reproductive endpoints. In fact, a dose of 1.0 μg 2,3,7,8-TCDD/kg bw given on GD8 produced responses similar to a dose of 0.2 μg 2,3,7,8-TCDD/kg bw given on GD15. In addition, the foetal concentrations of 2,3,7,8-TCDD measured on GD16 were reported to be very similar (Hurst *et al*., 2000). This would suggest that the critical determinant of these reproductive effects is the foetal concentration on GD15, which, as noted above, is likely to be higher following a single bolus dose on this day than that resulting from lower level chronic exposure. This weakens the relevance to human dietary exposure.

Many of the effects on the developing reproductive organs of rats described above have been confirmed by others (see Annex III). In all these studies bolus doses of 0.25 or 1 μg 2,3,7,8-TCDD/kg bw, administered on GD15, were used. The results of these studies also indicate that the Holzman rat is more sensitive to the effects of 2,3,7,8-TCDD than the Long-Evans rat.

Conclusion

There is sufficient evidence that prenatal 2,3,7,8-TCDD exposure of rodents produces a number of adverse effects on the developing male and female reproductive organs and their functions. The Committee found that 0.2 μg 2,3,7,8-TCDD/kg bw administered by gavage as a single bolus dose to pregnant rats at GD15 represents a LOAEL (and 0.05 μg 2,3,7,8-TCDD/kg bw a NOAEL) for induction of vaginal threads and alterations of the external genitalia in the female offspring of Long Evans rats (Gray *et al.*, 1997b). In the male offspring, the maternal dose of 0.05 μg 2,3,7,8-TCDD/kg bw represents a sensitive LOAEL in producing accelerated eye opening and a non-significant decrease in sperm counts (Gray *et al.*, 1997a), while 0.064 μg 2,3,7,8-TCDD/kg bw produced a significant reduction in ejaculated sperm count in the offspring of Holzman rats (Mably *et al.*, 1992c). Clear effects on several other parameters of male reproductive function, morphology and behaviour were seen in the Holzman rat at a dose level of 0.16 μg 2,3,7,8-TCDD/kg bw (Mably *et al.*, 1992a, b, c).

3.4.4. Effects on the immune system of the offspring of 2,3,7,8-TCDD-exposed rats

Gehrs and colleagues (1997a, 1997b) observed that 2,3,7,8-TCDD suppressed a delayed-type hypersensitivity (DTH) response to bovine serum albumin (BSA) in the 4-5 month old offspring of F344 rat dams that received 1 or 3 μg 2,3,7,8-TCDD/kg bw by oral gavage on gestational day 14 (GD14). In a subsequent study on the dose-response effects of 2,3,7,8-TCDD (Gehrs and Smialowicz, 1998, 1999) the DTH response to BSA was measured in the 4 and 14 month-old offspring of dams exposed by gavage to 0, 0.1, 0.3, or 1 μg 2,3,7,8-TCDD/kg bw on GD14. In the males, modest but significant suppression was observed at a dose of 0.1 μg 2,3,7,8-TCDD/kg bw at 14 months of age, while a maternal dose of 0.3 μg 2,3,7,8-TCDD/kg bw was necessary to cause suppression in the 14 month-old females. Both males and females were more sensitive to the suppression at 14 months of age than at 4 months of age.

Comments

The authors conclude that humoral immunity is less sensitive to 2,3,7,8-TCDD exposure than cell mediated immunity. In the study of Gehrs *et al.* (1997a) a cross-fostering experiment using a 2,3,7,8-TCDD dose of 1 μg/kg bw was included. While not mentioned in the results section of the paper, the authors state that the severity of the immune effects depended on the route of exposure, i.e. combined *in utero* and lactational exposure had more effect than lactational exposure only, which in turn had more effect than *in utero* exposure only. However, the authors note that in humans immunological development occurs earlier than in rats, therefore this observation may not be relevant to human risk assessment.

Conclusion

A study using pregnant F344 rats has demonstrated that prenatal exposure to single bolus doses of 2,3,7,8-TCDD of 0.1 μg 2,3,7,8-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 2,3,7,8-TCDD/kg bw (Gehrs *et al.*, 1997b; Gehrs & Smialowicz, 1998).

3.5. Evaluation of LOAELs for 2,3,7,8-TCDD exposure and the associated animal body burdens

In section 3.4 the following sensitive LOAELs for 2,3,7,8-TCDD in monkeys and rats were derived:

- Female monkeys fed 5 ng 2,3,7,8-TCDD per kg diet for 3.5 years and observed for another 10 years developed an increased incidence of endometriosis (Rier *et al*., 1993).

- A subtle non-persistent change in one, among several, parameters related to cognitive recognition (object learning) was reported in the offspring delivered after 16.2 and 36.3 months by the female monkeys fed 5 ng 2,3,7,8-TCDD per kg diet (Schantz & Bowman, 1989).

- Gavage administration of a single bolus dose of 50 ng 2,3,7,8-TCDD/kg bw to pregnant Long Evans rats on GD15 produced accelerated eye opening and a 25% (non-significant) decrease in sperm counts in their male offspring (Gray *et al*., 1997a).

- Gavage administration of a single bolus dose of 64 ng 2,3,7,8-TCDD/kg bw to pregnant Holzman rats on GD15 produced a significant reduction in sperm counts in their male offspring (Mably *et al*., 1992a, b, c).

- Gavage administration of a single bolus dose of 100 ng 2,3,7,8-TCDD/kg bw to pregnant F344 rats on GD14 suppressed a delayed type hypersensitivity response to bovine serum albumin in the male offspring at 14 months of age (Gehrs & Smialowicz, 1998, 1999).

The Committee noted that the bioavailability of 2,3,7,8-TCDD was not measured in any of the studies from which the LOAELs were derived. In a recently published study, Hurst *et al*. (2000) measured 2,3,7,8-TCDD concentrations in the tissues of pregnant Long Evans dams at GD16 following gavage administration at GD15 of 0.05, 0.2, 0.8 or 1.0 μg/kg bw, and reported the maternal body burdens as being 30.6 (60%), 97.4 (48%), 522.8 (65%) or 585.2 (59%) ng 2,3,7,8-TCDD/kg bw (percentage of dose), respectively. The Committee considered that this recent study by Hurst *et al*. (2000) provided an appropriate basis for the estimation of the bioavailability of 2,3,7,8-TCDD in pregnant rats at the dose levels associated with the sensitive LOAELs. The Committee therefore used an absorption rate of 60% for the gavage studies in pregnant rats. As regards absorption from the diet, the WHO Consultation (WHO, 2000) assumed an absorption of 50% from food for humans. Assuming that bioavailability from the dietary matrix is less than from a gavage dose, the Committee accepted this figure as being a reasonable approximation for the bioavailability of dietary 2,3,7,8-TCDD in the rhesus monkey study as well, given the fact that the absorption rate in gavage studies with rats was 60%.

The Committee also noted that, in the monkey studies, the daily intake of 2,3,7,8-TCDD was not reported. Several attempts have been made to estimate the intake of 2,3,7,8-TCDD by these monkeys, resulting in daily intakes ranging from 0.126 to 0.2 ng/kg bw/day (see Annex III). The Committee used the estimate of 0.151 ng 2,3,7,8-TCDD/kg bw/day published by DeVito *et al.* (1995), rounded to 0.15 ng 2,3,7,8-TCDD/kg bw/day. Assuming 50% absorption of 2,3,7,8-TCDD from the diet and a half-life for whole body elimination of 2,3,7,8-TCDD of 400 days in monkeys, as determined by Bowman *et al.* (1989), the Committee calculated that a daily intake of 0.15 ng/kg bw would produce a body burden of

25 ng/kg bw after 16.2 months and 37 ng/kg bw after 36.3 months in the study of reproductive development, and of 39 ng/kg bw after 42 months in the study of endometriosis.

In the Long Evans rat, accelerated eye opening and a non-significant reduction in sperm counts in the male offspring were recorded after a single maternal bolus dose of 50 ng 2,3,7,8-TCDD/kg bw (Gray *et al.*, 1997a). In the Holzman rat a maternal bolus dose of 64 ng 2,3,7,8-TCDD/kg bw was a LOAEL for reduction in sperm counts (Mably *et al.*, 1992c). These doses would correspond to body burdens of 30 and 38 ng 2,3,7,8-TCDD/kg bw, respectively, assuming 60% absorption.

Prenatal exposure of pregnant F334 rats to a single bolus dose of 100 ng 2,3,7,8-TCDD/kg bw on GD14 represents a sensitive LOAEL for persistent suppression of delayed type hypersensitivity in the male offspring (Gehrs and Smialowicz, 1998). This LOAEL would be associated with a body burden of 60 ng 2,3,7,8-TCDD/kg bw.

The estimated maternal body burdens from the studies with monkeys and rats are given in Table 3 along with the associated estimated human daily intakes (EHDI). These EHDIs are calculated using the formula given in Section 3.2.

TABLE 3. Estimated animal body burdens of 2,3,7,8-TCDD and associated estimated human daily intakes (EHDI).

Study	**Response at LOAEL**	**LOAEL**	**Maternal body burden* (ng/kg bw)**	**Associated EHDI (pg/kg bw)**
Schantz & Bowman, 1989	**Rhesus monkeys:** Subtle, non-persistent neurobehavioural changes (object learning) in offspring	0.15 ng/kg bw/day dietary administration	25-37¶	12.5-18.5
Rier *et al.*, 1993	**Rhesus monkeys:** Endometriosis	0.15 ng/kg bw/day dietary administration	39§	19.5
Gray *et al.*, 1997a	**Long Evans rats:** Accelerated eye opening and decreased sperm count in male offspring	50 ng/kg bw single bolus dose by gavage	30‡	15
Mably *et al.*, 1992c	**Holzman rats:** Decreased sperm count in offspring	64 ng/kg bw single bolus dose by gavage	38‡	19
Gehrs *et al.*, 1997b; Gehrs & Smialowicz, 1998	**F344 rats:** Immune suppression in offspring	100 ng/kg bw single bolus dose by gavage	60‡	30

*Increment over background. Background body burden in rats and mice is about 4 ng TEQ/kg bw (WHO, 2000).
‡Maternal body burden at gestational day 15.
¶Maternal body burden at delivery after 16.2 and 36.3 months of maternal exposure, respectively.
§Body burden at the end of dosing period (42 months).

As mentioned before, the Committee was not able to determine the clinical significance for humans, if any, of the findings in the study on neurobehavioural effects in the offspring of

2,3,7,8-TCDD-exposed monkeys. However, the Committee included the study in its evaluation because the estimated maternal body burden was within the range for which other sensitive, adverse effects have been documented.

3.6. Derivation of a tolerable intake of 2,3,7,8-TCDD and related compounds for humans

The studies discussed above provide LOAELs for the most sensitive adverse effects of 2,3,7,8-TCDD exposure in experimental animals. The Committee has calculated that these sensitive adverse responses were associated with exposure levels that produced body burdens between 25 and 60 ng 2,3,7,8-TCDD/kg bw.

The Committee calculated the associated estimated human daily intakes (EHDI) in the range of 12.5-30 pg 2,3,7,8-TCDD/kg bw. Such daily intakes are needed for a prolonged time period (20-30 years) to achieve these body burdens at steady state.

3.6.1. Extrapolation to humans

In order to arrive at a tolerable intake of 2,3,7,8-TCDD for humans an uncertainty factor needs to be applied. The uncertainty factor should account for the use of a range of LOAELs instead of a NOAEL, the possible differences between experimental animals and humans in susceptibility (toxicokinetics and toxicodynamics) to 2,3,7,8-TCDD and the potential interindividual variation in susceptibility (toxicokinetics and toxicodynamics) to 2,3,7,8-TCDD within the human population.

- As the LOAELs reported for these sensitive endpoints were considered to be close to the NOAELs and representing marginal effects the Committee found it appropriate to use a factor of 3 to allow extrapolation to NOAELs.
- 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.
- In considering aspects of the variability in the toxicokinetic properties of 2,3,7,8-TCDD in humans, the Committee noted that reported mean half-lives of 2,3,7,8-TCDD in humans vary from 5.1 to 11.3 years (IARC, 1997). The distribution of these mean half-lives shows a mean of 8 years and a standard deviation of 2.1 years. Using the mean plus two standard deviations (12.1 years) to describe the 95% upper interval for the half-life of 2,3,7,8-TCDD in humans a data-derived uncertainty factor of 1.5 (12.1/8) would be predicted for interindividual variations in toxicokinetics among humans. However, the human data were mainly obtained from occupationally exposed men, therefore the variability in toxicokinetics among females is not adequately covered. As all the most sensitive effects of 2,3,7,8-TCDD were seen after 2,3,7,8-TCDD exposure of female animals the Committee found it most appropriate to use the default uncertainty factor of 3.2 as recommended by WHO (WHO, 1994) to account for interindividual variations with regard to absorption, biotransformation, accumulation and elimination of 2,3,7,8-TCDD within the human population.
- With regard to the potential differences in toxicodynamics between experimental animals and humans and within the human population, studies of Ah receptor binding affinity and adverse responses directly dependent on Ah receptor activation suggest that humans are

less sensitive to 2,3,7,8-TCDD than responsive rodent strains. However, studies of some biochemical or cellular effects, such as CYP1A1 and CYP1A2 induction, suggest a comparable sensitivity. Therefore, for some endpoints it can not be excluded that the most sensitive humans might be as sensitive to the adverse effects of 2,3,7,8-TCDD as experimental animals. The Committee concluded that no uncertainty factor needs to be applied for differences in toxicodynamics between experimental animals and humans and for interindividual variation among humans.

Therefore, the Committee considered an uncertainty factor of 10 (3*3.2) adequate for the protection of human health from exposure to 2,3,7,8-TCDD.

3.6.2. Tolerable intake

The Committee considered the studies showing the most sensitive effects of 2,3,7,8-TCDD in animals (Table 3).

The Committee has identified the limitations in the estimation of body burdens in the studies listed in Table 3, and consequently in the associated estimated human daily intakes of 2,3,7,8-TCDD derived from these studies. The Committee was unable to identify any single study as being sufficient, by itself, to provide a firm basis for the establishment of a tolerable intake. It therefore considered that the studies identified in Table 3, providing EHDIs in the range of 12.5 to 30 pg 2,3,7,8-TCDD/kg bw, and within the limits of precision of the estimates, all contribute to the derivation of a tolerable intake. Applying the 10-fold uncertainty factor established in Section 3.6.1 to these EHDIs suggests a tolerable intake in the range 1 to 3 (rounded figures) pg/kg bw/day.

There are currently no scientific data to guide the Committee on selection of a single value from the range of 1 to 3 pg 2,3,7,8-TCDD/kg bw/day. However, because of the acknowledged uncertainties the Committee concluded that the lower end of the range, i.e. 1 pg/kg bw/day, should be considered as a temporary tolerable intake.

Recognising that compounds like 2,3,7,8-TCDD and related substances have very long half-lives in the human body, the Committee found it more appropriate to express the tolerable intake on a weekly rather than a daily basis. Therefore the Committee established a temporary tolerable weekly intake (t-TWI) of 7 pg 2,3,7,8-TCDD/kg bw.

The Committee extended the t-TWI to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs, expressed as WHO TEQ (see Section 3.6.3).

3.6.3. Extension of the temporary tolerable weekly intake (t-TWI) for 2,3,7,8-TCDD to include all 2,3,7,8-substituted PCDDs and PCDFs and dioxin-like PCBs

In order to extend the t-TWI for 2,3,7,8-TCDD to all 2,3,7,8-substituted PCDDs and PCDFs and dioxin-like PCBs, expressed as WHO-TEQ, it was considered essential that the differences in half-lives between 2,3,7,8-TCDD and the other dioxins and dioxin-like PCBs are either small or accounted for in the establishment of the WHO TEF values as proposed by van den Berg *et al.* (1998).

The most critical aspect of the use of TEFs is the possibility that the half-life of an individual congener in the human body may be sufficiently longer than that of 2,3,7,8-TCDD itself to result in a greater body burden of that congener than would be expected on the basis of the half-life of 2,3,7,8-TCDD. If the TEF for that congener was similar to that of 2,3,7,8-TCDD

and the difference in half-life was not accounted for by the TEF the effective toxicity of the congener could be greater than that predicted by the calculated TEQ.

The converse situation, in which the half-life of a congener is shorter than that of 2,3,7,8-TCDD itself, would result in the effective toxicity of that congener in a mixture being less than its calculated TEQ. Therefore, in this instance the use of the assigned TEF would provide a greater margin of safety and would be the more conservative option in human risk assessment.

The Committee therefore examined available studies on half-lives of dioxins in humans and information on dioxin-like PCBs in animals in order to identify congeners with half-lives greater than 2,3,7,8-TCDD and subsequently consulted the WHO TEF derivation to examine whether this aspect had been covered.

Only few studies have attempted to determine the half-life in humans of a wide range of the 2,3,7,8-substituted PCDDs or PCDFs. The two that provide the best information are those of Flesch-Janys *et al*. (1996) and of Rohde *et al*. (1999). Both of these utilised the same cohort of workers that had been exposed occupationally to high levels of these compounds.

The congeners of concern were 1,2,3,7,8-PnCDD with a half-life in humans of the order of 14 years and a TEF of 1 and 2,3,4,7,8-PnCDF with a half-life in humans of the order of 16 years and a TEF of 0.5. These congeners, in particular 2,3,4,7,8-PnCDF, may provide an appreciable proportion of the TEQ associated with exposure to some PCDD and PCDF mixtures. Most other congeners had shorter half-lives than 2,3,7,8-TCDD.

As judged from limited information 1,2,3,7,8-PnCDD and 2,3,4,7,8-PnCDF also have longer half-lives (*ca* 30 and 60 days, respectively) in rats than 2,3,7,8-TCDD (*ca* 20 days) (van den Berg *et al.*, 1994). The Committee noted that the TEF values for 1,2,3,7,8-PnCDD and 2,3,4,7,8-PnCDF have been established on the basis of liver tumour promotion studies in rats and 3-month studies of toxicity in rats and mice in which the chemicals were at or approaching steady state conditions. In these studies 2,3,7,8-TCDD was included as a reference compound. Thus for 1,2,3,7,8-PnCDD, WHO in 1997 (van den Berg *et al.*, 1998) recommended that the previously used TEF of 0.5 was increased to 1.0 based on new *in vivo* tumour promotion data and CYP1A1/CYP1A2 induction potencies from subchronic studies. For 2,3,4,7,8-PnCDF, most relative potencies dealing with relevant toxic endpoints from subchronic studies supported the continued use of a value of 0.5.

Among the dioxin-like PCBs the only one that has a sufficiently high TEF value to warrant consideration is 3,3´,4,4´,5-PnCB (PCB 126). However, the WHO TEF value of 0.1 has been established on the basis of a number of *in vivo* endpoints in subchronic studies in rats. Thus, any differences in half-lives of congeners from that of 2,3,7,8-TCDD itself appear to be accounted for.

Based on the above considerations, the Committee concluded that the t-TWI for 2,3,7,8-TCDD could be extended to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs, and established a group t-TWI of 7 pg WHO TEQ/kg bw for these compounds.

The Committee also concluded that the group t-TWI is to be expressed in WHO TEQs and recommends the use of the WHO TEF approach for expressing the intake in humans of

PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in units of 2,3,7,8-TCDD equivalents (TEQs).

In line with the WHO Consultation the Committee recognised that a number of uncertainties exist in the use of the TEF concept for human risk assessment. However, pragmatically it remains the most feasible approach. Use of 2,3,7,8-TCDD alone as the only measure of exposure to PCDDs, PCDFs and dioxin-like PCBs would severely underestimate the risk to humans from exposure to these compounds.

3.6.4. Related compounds

The Committee considered that in the future there might be a need to include related compounds, such as brominated and mixed chlorinated/brominated congeners in the TEF approach. However, only a limited database is currently available on these compounds.

The Committee discussed a proposal that hexachlorobenzene (HCB) should be classified as a dioxin-like compound, with a TEF value of 0.0001 (van Birgelen, 1998). However, the Committee questions the validity of using a TEF approach for HCB. While HCB is a persistent chemical that accumulates in the food chain and also is able to bind weakly to the Ah receptor, its structural resemblance with PCDDs and PCDFs is, at best, weak. Although 2,3,7,8-TCDD and HCB can affect the same physiological systems and share some target organs of toxicity, the effects produced in these systems or organs differ. Therefore, with respect to the toxic responses to HCB and dioxins it is also questionable that the combined effects are dose or concentration additive. These considerations led the Committee to conclude that, on the basis of current knowledge, the WHO TEF approach is not justified for HCB.

4. Risk characterisation

The average dietary intakes of PCDDs and PCDFs for adults of various European countries were estimated to be between 0.4-1.5 pg I-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. Thus, the total intake of dioxins and dioxin-like PCBs from the diet is 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 will exceed the group t-TWI of 7 pg WHO-TEQ/kg bw derived by the Committee.

The currently estimated average human intakes in European countries of 1.2-3.0 pg WHO TEQ/kg bw/day would produce body burdens of 2.4-6.0 ng WHO TEQ/kg bw (see Section 3.2). A steady state body burden of 2 ng WHO TEQ/kg bw would be produced at an intake at the group t-TWI.

The Committee emphasises that it did not perform a quantitative assessment of the health risk associated with exposure to dioxins and dioxin-like PCBs. This is because the available data on high dose animal studies and studies of human occupational and accidental exposures cannot be extrapolated quantitatively with any confidence down to values corresponding to the background exposures of the general population. Instead, the Committee established a group t-TWI and noted that a TWI is not a lower bound of toxicity, it is an estimate of a safe level of intake and is derived conservatively using uncertainty factors applied to NOAELs or LOAELs. However, that does not necessarily mean that there is an appreciable risk to the health of individuals exceeding slightly the group t-TWI, but exposure above the tolerable weekly intake leads to an erosion of the protection embedded in the group t-TWI.

Various risk assessments of PCDDs, PCDFs and dioxin-like PCBs have identified groups of the population that may experience higher than average exposure through high consumption of heavily contaminated food, human milk (breast-fed infants), or occupational exposure. It is important to note that the sensitive endpoints that drive the derivation of the t-TWI relate to the body burden of dioxin in fertile women. Except for promotion of endometriosis, the group at risk is the unborn foetus, the exposure of which depends on the mother's body burden. It also has to be recognised that, for the general population, this body burden is the result of dioxin exposure *via* the food over many years. The long half-lives in humans of the compounds involved mean that the steady-state body burdens usually reflect a stable situation in which brief exposures above background will not result in changes.

Meat, eggs, milk, farmed fish and other food products may be contaminated above background by dioxins from feedingstuffs. Such contamination may be due to a high level of local environmental contamination, for example from a local waste incinerator, or to incidents, such as the Belgian episode, where animal feedingstuffs were contaminated, or to a high content of dioxins in fishmeal and fish oil. Wild fish from certain polluted areas may be highly contaminated.

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. The intake by the breast-fed infants was mimicked in the studies that were considered during the derivation of the t-TWI, in which the offspring was exposed through the suckling phase.

In this context, the Committee reiterated the conclusions of the WHO meetings on the health significance of contamination of human milk with dioxins and PCBs, namely that the current

evidence does not justify altering recommendations on the promotion of, and support for, breast-feeding (Brouwer *et al*., 1998).

The Committee welcomed the clear evidence of a decrease within the last 10 years in dioxin levels in foods and human milk in almost every region for which suitable data exist. This can be attributed most probably to the enhanced identification and control of input to the environment. The relationship between average dietary exposure to dioxins and the resulting tissue levels in humans is illustrated by data from Germany which showed that decreases in average TEQ intake over the course of 7 years (1989-1996) resulted in similar declines in the concentrations in human milk and blood.

5. Risk management strategies

5.1. Primary approaches to reduce human dietary exposure to dioxins and dioxin-like PCBs

Due to their comparable toxicological properties the Committee included the dioxin-like PCBs (non-*ortho* and mono-*ortho* PCBs) in the risk assessment of the dioxins. The risk management should therefore also include the dioxin-like PCBs.

Dioxins are not produced intentionally and, unlike pesticides, are not applied to plants or farmland for beneficial purposes, but are produced as unwanted by-products in a number of industrial and thermal processes. Therefore, the measures to achieve a decline of the dioxin levels in the environment must primarily focus on the identification of relevant new sources as well as on the reduction of emissions from well-known sources to levels as low as technically achievable. Such actions must be part of an ongoing process. Positive effects of any measures taken today will not be seen immediately but, because of the persistent nature of dioxins and dioxin-like PCBs, only after a few years. This has been demonstrated in various countries which, at the end of the 80s, took a number of measures to reduce the dioxin emissions and to lower their levels in the environment. These measures included *inter alia*, optimisation of incineration technology, bans on the production and use of pentachlorophenol (PCP) and certain other chemicals, phasing out of leaded petrol with halogenated scavengers or substitution of chlorine in paper pulp bleaching with other reagents. Moreover, several risk reduction measures have been undertaken concerning the production and use of PCBs since the late 70s. Their success is demonstrated by a significant reduction of dioxin and PCB contamination of the environment, feed and particularly of food and human milk in several countries. Investigations of food and human milk samples from Finland, Germany, The Netherlands and the United Kingdom for example, revealed dioxin levels which are approximately 50% lower compared to corresponding samples collected and analysed about 10 years ago. As a result of the control measures, the average daily dioxin intake for adults was reduced from around 2 pg I-TEQ/kg bw to below 1 pg I-TEQ/kg bw. However, this value is approximately doubled when the dioxin-like PCBs are included. This shows that, even in those countries which took numerous measures to minimize dioxin emissions, the average intake of dioxins and dioxin-like PCBs may exceed the t-TWI value for dioxins and dioxin-like PCBs of 7 pg WHO-TEQ/kg bw as derived by the Committee.

The Committee therefore recommends that continuing efforts should be made to limit environmental releases of dioxins and related compounds to the lowest levels that are feasible. This is the most efficient way to reduce the presence of dioxins and analogues in the food chain and to ensure continued reductions in human body burdens. In this context it should be noted that recent investigations of human milk (Fürst, 2000) and blood (Päpke, 2000) apparently indicate that the dioxin levels are no longer declining.

5.2. Secondary approaches to reduce human dietary exposure to dioxins and dioxin-like PCBs

5.2.1. Maximum levels

If measures to reduce the human body burden significantly were to be based exclusively on maximum levels for dioxins and dioxin-like PCBs in different food commodities, such as

dairy products, beef, pork, poultry, eggs or vegetables, these levels would have to be below present mean background contamination of the respective foodstuffs. However, the consumption of food with average contamination levels already results in a weekly intake that is in the range of, or exceeds, the t-TWI value for dioxins and dioxin-like PCBs. Therefore, setting maximum levels that will considerably reduce human exposure would result in a considerable part of the present food supply being declared unfit for human consumption. On the other hand, a positive consequence of the setting of maximum levels on food commodities would be the implementation of regulatory control systems.

5.2.2. Action thresholds and target values

A more feasible approach seems to be to set action thresholds and target values for those food groups which contribute most to the human body burden, linked with measures to reduce emissions. This would need to be accompanied by appropriate compulsory and well planned monitoring control programmes. In this context, target values indicate the average levels of contamination of food products required to bring the exposure down to the TWI value. An action threshold is a level of contamination that triggers specific risk management actions. Action thresholds are higher than target values.

Action thresholds could be set, for example, using appropriate percentiles (e.g. 90th, 95th or 99th percentile) of the distribution of the contamination levels of the respective food commodity. Another approach is the use of confidence limits which are less influenced by very high levels of contamination and therefore are good descriptors of the data distribution even if the distribution is not fully known.

If action thresholds are exceeded, efforts should be made to identify the specific source and/or pathways responsible for the contamination. Once identified, measures should be defined and applied to prevent or to reduce contamination from the source. Moreover, it should be decided case by case whether a specific food should be withdrawn from the market. This would remove the foods with the highest contamination from human consumption. The effect for the general population will presumably be minimal, but this measure will protect at least those people who often consume the particularly highly contaminated food items.

Target values would normally be lower than the actual average background levels for the respective food commodity and therefore can only be reached after a further reduction of the emissions of dioxins and dioxin-like PCBs into the environment. These values can be estimated on the basis of the contribution of each food item to the total intake based on the actual levels. From this, assuming the same consumption habits, one can deduce the target levels which are necessary in order to reach the t-TWI value for dioxins and dioxin-like PCBs. Consequently, target values will be the driving force behind measures necessary for a further reduction of emissions into the environment. With increasing decline of emissions, the distribution of the contamination levels for the different food groups will show a shift to lower levels and will slowly come closer to the target values. As a result of this, the action thresholds might be revised after a certain period of time.

The Committee notes that until now, there is no legal basis in the EU for setting action thresholds and target values in food, as Council Regulation 315/93 laying down Community procedures for contaminants in food only provides the possibility for establishing maximum limits for contaminants after following a specific procedure. However, considering that the dioxins and dioxin-like PCBs are not applied intentionally to food and taking into account the importance of food being the main source for human exposure to these compounds, it seems

worthwhile to reconsider the conventional approach of setting maximum limits in the EU and to provide a legal basis for a wider range of possible measures to limit the presence of contaminants in food.

5.3. Implementation of monitoring programmes

In order to get a broader knowledge of the distribution of dioxins and dioxin-like PCBs in the EU, it seems mandatory to include these substances in harmonised monitoring programmes. This could be performed, for example, as part of the co-ordinated control programmes or national residue control plans which are already conducted in all Member States for other compounds, such as pesticides and veterinary drugs. Those Member States which currently do not analyse these substances on a routine basis might require scientific support to set up appropriate analytical programmes.

5.4. Non-dioxin-like PCBs

Although their manufacture, processing and distribution is prohibited in almost all industrial countries since the late 80s, a huge amount of PCBs is still in existence. According to Council Directive 96/59/EC of 16 September 1996 on the disposal of polychlorinated biphenyls and polychlorinated terphenyls (PCB/PCT), the deadline to take all PCB-containing equipment out of service in all Member States expires by the end of 2010 at the latest. Therefore, in accordance with Council Directive 75/442/EEC of 15 July 1975 on waste, appropriate measures must mainly aim at avoiding the abandonment, dumping or uncontrolled disposal of waste and the use of processes or methods which could harm the environment. In order to determine whether PCBs have entered into the environment due to improper disposal practices or whether leaks from equipment have occurred, randomly collected food samples should be analysed routinely.

Due to their comparatively lower toxicity, non-dioxin-like PCBs were given a lower priority by the Committee for the time being. On the other hand, the exposure of the general population to these congeners comes mainly from food (IPCS, 1993). Therefore, the risk assessment on non-dioxin-like PCBs should be prepared in the near future, in order to provide the scientific basis for management decisions. As a prerequisite for this task, the current risk assessment for PCBs should be updated. This reassessment should preferably be based on a congener-specific toxicological evaluation.

6. Conclusions

- The Committee established a temporary tolerable weekly intake (t-TWI) of 7 pg 2,3,7,8-TCDD/kg body weight (bw), using the body burden approach.

- The t-TWI is based on the most sensitive endpoints reported in animal studies. These were developmental and reproductive effects in rats and monkeys and an increase in the incidence of endometriosis in monkeys. It would also adequately protect against the carcinogenic effects of 2,3,7,8-TCDD, which require substantially higher body burdens and for which a threshold approach is applicable due to its non-genotoxic nature.

- The Committee concluded that the t-TWI for 2,3,7,8-TCDD could be extended to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs, and established a group t-TWI of 7 pg WHO TEQ/kg bw for these compounds.

- The average human dietary intake of PCDDs, PCDFs and dioxin-like PCBs in European countries has been estimated to be 1.2 to 3.0 pg WHO TEQ/kg bw/day.

- More than 90% of human dioxin exposure derives from food; food of animal origin normally contributes to about 80% of the overall exposure.

- Efforts in limiting the release of dioxins have been successful in various European countries. However, a considerable proportion of the European population will still exceed the group t-TWI. Therefore, continuous efforts should be made to limit environmental release of PCDDs, PCDFs and dioxin-related compounds.

7. Recommendations

- To reconsider the current conventional approach of setting maximum limits in the EU and to provide a legal basis for a wider range of possible measures (e.g. by setting action thresholds and/or target values) to limit the presence of contaminants in food.

- To collect regularly and adequately data on the occurrence of dioxins and dioxin-like PCBs in foods in order to follow temporal trends in the exposure of populations of different EU Member States. The occurrence data must be based on samples representative of foodstuffs consumed in the EU Member States.

- To collect food consumption data on a EU scale from national surveys carried out on a regular basis. Particular attention must be given to the improvement of comparability of national food intake data.

- To include dioxin-like compounds in harmonised monitoring programmes in order to get a broader knowledge on the distribution of these compounds across the EU.

- To apply the WHO-TEF approach for expressing occurrence and dietary exposure data for PCDDs, PCDFs and dioxin-like PCBs in toxic equivalents of 2,3,7,8-TCDD (TEQ).

- To continue with the regular collection of data on levels of dioxin-like compounds in human milk and blood in order to follow temporal trends in the body burden of populations across the EU.

- To obtain data appropriate for the determination of TEFs for related compounds, such as brominated and mixed chlorinated/brominated congeners.

- To continue the biological and toxicological research on dioxins and dioxin-like PCBs, which may help to refine the risk assessment.

- To update the current risk assessment for non-dioxin-like PCBs in view of their predominance in foods. The assessment should be preferably based on a congener-specific basis.

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ANNEX I

STATISTICAL ASSESSMENT OF PCDD, PCDF, AND DIOXIN-LIKE PCB LEVELS IN EUROPEAN FOODSTUFFS

Table of Contents

1. Introduction

On June 7, 2000, the EU SCOOP (Task 3.2.5) report entitled "Assessment of Dietary Intake of Dioxins and Related PCBs by the Population of EU Member States", was released. The project had been carried out at the request of the Commission of the European Communities, to gather information on dietary exposure to PCDDs, PCDFs, and dioxin-like PCBs in the participating countries (Belgium, Denmark, Finland, France, Germany, Italy, The Netherlands, Norway, Sweden, and the United Kingdom).

The Committee examined whether the EU SCOOP database was adequate to evaluate the general level of exposure of the population in the participating countries. In doing so, it analysed the occurrence of dioxin and dioxin-like PCB contamination levels in the different foods; concurrently, examples suitable for risk management were prepared.

Therefore, the polychlorodibenzo-*p*-dioxin (PCDD), polychlorodibenzofuran (PCDF), and dioxin-like polychlorobiphenyl (PCB) "raw" data contained in Annex 3 of the EU SCOOP report were statistically treated to derive descriptors useful to characterize the contaminant occurrence in the analised foods.

2. Structure of pertinent EU SCOOP data — caveats

PCDD, PCDF, and dioxin-like PCB data were gathered in the EU SCOOP report starting in 1982. However, this assessment is concerned with the data since 1995 only, to more closely reflect today's current levels of food contamination. All concentration values were originally expressed in cumulative TE units or TEQs (i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity equivalents), derived from the application of the international or NATO/CCMS toxicity equivalency factor system (I-TEFs) to PCDD and PCDF analytical results (NATO/CCMS, 1988); for PCBs, the former WHO-ECEH toxicity equivalency factors (PCB-TEFs) of Ahlborg *et al*. (1994) were used. These units have been retained in this assessment.

In the EU SCOOP report, the dioxin-like PCB values were classified into subsets identified as "nor_PCBs", "mor_PCBs", "All_PCBs", and "ICES7", depending on the specific cluster of congeners determined. In the period under consideration "mor_PCBs" were absent at the start. In addition, the scant "ICES7" values were left out to increase the congruity of data sets and subsets. Therefore this assessment was based on the "All_PCBs" and "nor_PCBs" subclasses only. As some of the TE results used were originally obtained by quantitating only a limited

number of the dioxin-like PCB congeners, the corresponding values used here would be affected by some underestimation.

Original analytical results appear to have been obtained largely without adequate harmonisation of analytical procedures and/or intercalibrating processes of the laboratories in the different countries. This would possibly influence the comparability of results. For the dioxin-like PCBs, the difficulty of making comparisons is also increased as the selection of the PCBs measured varied considerably from study to study. In addition, some data may be affected by a slight overestimation as the EU SCOOP report generally adopted the "upperbound approach", that is the value of the limit of determination was used in calculating TE values for those congeners that were not quantitated.

For this assessment, the data of the EU SCOOP report were treated as if they were of comparable analytical quality. In particular, no distinction was made as to how determination thresholds (limits of quantification -LOQs- or limits of detection -LODs-) were entered in the original calculations to yield the available TE values: however, it was assumed that the general conclusion of the study would not be significantly influenced.

Most concentration data appear to have been provided by the participating countries after pooling of samples at the source or averaging of analytical results (i.e., more than one sample $\rightarrow$ one result). Consequently, the number of data available (or "true observations", N1) is actually less than the number of physical samples (N2) that they represent. For the same reasons, the EU SCOOP database cannot account for the effective multiplicity underlying the database itself and the correlated data sets (see Section 4).

3. Procedure and guidance for interpretation of results

The statistical descriptors resulting from the present assessment have been summarised in the following self-explanatory tables, concerning:

PCDDs and PCDFs

Table 1.	The original weighted and unweighted values.
Table 1bis.	The weighted and unweighted log-transformed values.
Table 2.	The log-transformed weighted values, estimates reverted to linear field.

Dioxin-like PCBs

Table 3.	The original weighted and unweighted values.
Table 3bis.	The weighted and unweighted log-transformed values.
Table 4.	The log-transformed weighted values, estimates reverted to linear field.

Each set and subset of the EU SCOOP database was first characterised by estimating the mean (<X>), median (MED), and standard deviation (SD) of original data (Tables 1 and 3); accordingly, the range covered by the data was also reported. However, the relevant statistical descriptors in Tables 2 and 4 were estimated after a log-transformation passage (Tables 1bis and 3bis) that, in general, improved the normality of the original distributions. This was observed in particular when primary, unweighted data sets and subsets (N1) were used (Shapiro and Wilks' *W* test). A comparison of the mean values reported in Tables 2 and 4 with the weighted <X> of Tables 1 and 3, respectively, provides an indication of the effect of the log-transformation passage. The BMDP statistical software package was employed for statistical analysis.

For mean (<X> or μ) and standard deviation (SD or σ) weighted estimates, weighting was obtained by utilising the original sampling frequency (N2), reflecting the physical reality. However, the degrees of freedom used to estimate confidence intervals (CIs) of weighted data sets and subsets were determined from the true observations N1.

Table formats were laid out to accommodate the assessment outcome of the PCDD and PCDF data set (larger than that of PCBs). Furthermore, to have a better insight into features of the contamination and to provide more elements for intragroup comparisons, the data originally clustered in the EU SCOOP database under a primary food group (e.g., *Meat and meat products*) were divided, where feasible, into subsets associated with more homogeneous food subgroups. On the whole, with reference to Tables 2 and 4 in particular, the following points may be observed (unless otherwise indicated, the available PCB results were insufficient for statistics).

Cereals and cereal products — A poorly characterised food group. A food item ("pastry") exhibited higher contents of lipids and, relative to other cereal items, PCDDs and PCDFs. Thereafter, two subgroups were identified with a lipid content cut-off at 2%. The subgroup with <2% lipids was made of bread, rice, pasta, and rye and wheat flours.

Eggs — As concerns PCDDs and PCDFs, this was a food group for which there was sufficient data available.

Fats and oils — An insufficiently characterised food group on both issues, the quality of the matrices and the contamination levels.

Fish and fish products — A preliminary statistical assessment showed the existence of a significant overlapping of PCDD and PCDF concentrations between the different types of wild fish (Mann-Whitney test). Therefore, the group was subdivided into WILD FISH, farmed FRESHWATER FISH, and OTHERS. Farmed freshwater fish was made into a different subgroup by *tentatively* integrating the entries clearly so qualified in the EU SCOOP report with those not specifically identified but expected to come from farms. On average, relative to WILD FISH the FRESHWATER FISH subgroup appears to exhibit a tendency to decreased contamination levels and a smaller range of values (this effect would be more pronounced for the dioxin-like PCBs than for the PCDDs and PCDFs). However, the differences between the wild and freshwater fish data for both groups of analytes are not significant. Therefore, in consideration of the specific risk management measures that might be developed for fish farming, an *ad hoc* investigation seems to be required to collect additional clarifying data. In the WILD FISH subgroup, the freshwater fish contribution is due to three entries ("carp", "catfish", and "pike"; N1 = 3; N2 = 64). In OTHERS only "cod liver oil" and "fish oils" are present (N1 = 2; N2 = 15). In general, the number of PCB results available in the whole data set and by the subset was sufficient for statistics.

Fruit and vegetables — As concerns PCDDs and PCDFs, this was a food group for which there was sufficient data available.

Human milk — As concerns PCDDs and PCDFs, this was a food group for which there was sufficient data available.

Meat and meat products — This food group was made up of quite different food types, from farmed animals to game. In addition, as some of the items reported in the EU SCOOP report were rather specific ("liver") or unqualified ("mixed meat"), the entire group was broken

down into six subgroups. Given the usefulness of having a detailed statistical outcome by the food type, it can be observed that the data subsets associated with POULTRY, BEEF AND VEAL, and "mixed meat" have similar mean levels of PCDDs and PCDFs. The corresponding mean estimate for PORK meat is approximately half of those reported for the other three subgroups; however, these differences are not statistically significant. GAME and "liver" data subsets are characterised by PCDD and PCDF contamination levels that are statistically different from the remaining subsets (Mann-Whitney test). The number of PCB results available in the whole data set was sufficient for statistical analysis, whereas the PCB data in the subsets were, in general, insufficient.

Milk and milk products — This food group was made of two subgroups: one containing data exclusively pertaining to milk (MILK AS SUCH); the other containing data concerning cheese, butter, and two unspecified dairy materials (OTHERS). These subgroups have somewhat different mean contamination levels, within a factor of 2 for both groups of analytes; however, the differences are not significant. The statistical descriptors of PCDD and PCDF data reported in the tables for the entire group and for the MILK AS SUCH subgroup were obtained by eliminating analytical results associated with milk specimens qualified as "industrial" type (N1 = 7; N2 = 82): in fact, in at least three cases out of seven the latter exhibited quite high PCDD and PCDF concentrations, ranging from 19 to 62 pg I-TEQ/g, lipid basis. This specific group of samples was found to contribute by some 15 and 4%, respectively, to the unweighted and weighted MILK AS SUCH data subsets. The number of PCB results available in the whole data set and by subset was in general sufficient for some statistical analysis; in contrast to the dioxins, the number N1 of "industrial"-type specimens outweighed that of specimens not affected by industry impact and used for statistical analysis.

4. Dioxin and dioxin-like PCB contamination distribution patterns — elements for risk management

In order to provide examples for risk management, the theoretical frequency distributions of contamination levels were derived for a number of food items on the basis of the weighted mean (μ) and standard deviation (σ) values estimated in Tables 1bis and 3bis for the log-transformed dioxin and dioxin-like PCB data respectively. The exercise was focused on foods of animal origin due to their relevance to the issue; in addition, only food items characterised by a reasonably high number of true observations (N1 >10) were considered. With reference to Figures 1-3, these distributions are discussed below.

Each pair of statistical descriptors (μ, σ) was first used to derive the pertinent log-Normal distribution through the function $F(X; \mu, \sigma)$, where X (>0) is the "log-Normal" random variable. Then, the values of the log-transformed concentrations, corresponding to the percentile or quantile values chosen for the exercise ($Q(.95)$ and $Q(.99)$), were estimated with a readily available statistical software package such as Microsoft Excel: the operation performed was equivalent to integrating $F(X; \mu, \sigma)$ between the lower end of the range and a certain value of X to obtain the sought area fraction, X being expressed in the appropriate concentration units (i.e., pg I-TEQ/g or pg PCB-TEQ/g, lipid basis).

Thereafter, the log-Normal frequency curves obtained were converted to a linear field, as were all the relevant estimates (Figures 1-3). Seven frequency functions were available for PCDDs and PCDFs (Figures 1 and 2); however, only two were described for dioxin-like PCBs (Figure 3) due to the limited number of data available. It should be pointed out that

estimation of percentiles could not be carried out through the convenient technique of numerical interpolation due to the highly variable form of the original data.

With reference to Figures 1 and 3, the following general points may be made. The data sets of PCDDs and PCDFs in eggs and milk (and its products) appear to cover ranges of reduced spread. It is apparent that both eggs and milk come exclusively from farming, this involving a relatively limited number of productive species and feeding habits generally controlled by humans. On the contrary, dioxin and dioxin-like PCB data in fish spread over two to three orders of magnitude (compare also with the data ranges in Tables 1 and 3). This is readily explained by the large variety of organisms analysed, including farmed as well as wild fish, fish from various regions of the world, and fish belonging to different levels of the trophic web up to high level predators, these latter often showing the effects of very pronounced biomagnification processes. For meat, mostly produced through farming and exhibiting an intermediate spread of the data, the extreme values to the right are associated with a few unusually high dioxin levels in pork meat (Figure 2) and with minor contributors such as liver and game. However, both fish and meat are characterised by severely skew distributions of dioxin and dioxin-like PCB concentrations. This reflects the remarkable dispersion of the contamination values found in the two food groups.

In the figures, five numerical values (X_{min}, $<X>$, the values associated with the 95th and 99th percentiles (respectively, $Q(.95)$ and $Q(.99)$ estimates), and X_{max}) have been superimposed on the frequency distribution pattern for the appropriate food item. However, for dioxin-like PCBs in fish and meat (Figure 3), the $Q(.99)$ estimate appears to exceed the X_{max} value, whereas for PCDDs and PCDFs in beef and veal (Figure 2) both percentile estimates exceed X_{max}. As mentioned above, this may be explained by the fact that the theretical frequency distribution patterns obtained were constructed from data sets essentially containing “means” (more than one sample $\rightarrow$ one result, from either averaging single results or pooling samples at the origin) rather than individual results (one sample $\rightarrow$ one result). In other words, potential extreme concentration values at both ends of the distributions would be expected to be absent as they are already included in the means presented by the EU SCOOP database.

The estimated $Q(.95)$ and $Q(.99)$ values shown in the figures are only intended to provide examples of how to use the statistical descriptors (namely, the above mentioned μ and σ) to derive the frequency distribution patterns of contaminants in different foods, and from these to identify cut-off points for risk management. It may be recalled that a concentration value associated with a given percentile (e.g., the 95th) divides the data into two subsets: 95% of the data will fall below that level, whereas the remaining 5% will be higher.

Therefore, if we look at the frequency distribution patterns in Figure 1, a dioxin cut-off value for acceptance set, for example, at 2 pg I-TEQ/g, lipid basis, would keep on the market 95% of the eggs, *almost* 95% of the milk (and its products), and *almost* 95% of the meat. However, it would keep most of the fish and fish products out of the market (a cut-off value of about 80 pg I-TEQ/g would be the appropriate value for the percentile level chosen).

As a higher percentile level (e.g., the 99th) tends to exclude less of the food material with a higher contamination and therefore save more of it for the market, this type of choice would be less conservative toward the consumers’ health than that of the 95th percentile. Selecting a low percentile, such as $Q(.50)$, would have a tremendous impact on both the consumers’ exposure and the market. As a last observation, the magnitudes of $Q(.95)$ and $Q(.99)$ values for a given frequency distribution may vary considerably relative to each other (e.g., for eggs

the *Q*(.99) estimate is only 25% higher than the value associated with *Q*(.95), whereas for fish the difference is over 100%).

Although the PCDD and PCDF data subsets associated with poultry, beef and veal, and pork meat subgroups are not statistically distinguished, the specifically correlated frequency distribution patterns are illustrated in Figure 2 to provide an additional example for hypothetical risk management measures at the level of the food subgroups. It may readily be observed that the 95th percentile is now considerably lower than the cut-off value for acceptance of 2 pg I-TEQ/g, lipid basis, referred to above: in fact, the *Q*(.95) estimate appears to be in the order of 1 pg I-TEQ/g, or even lower for pork meat (approximately, 0.6 pg I-TEQ/g). As to the latter, from the EU SCOOP database the long tail to the right of the distribution appears to be essentially due to a few particularly high concentration values, whereas most of the data fall below 0.5 pg I-TEQ/g.

Similar considerations may be applied to the dioxin-like PCBs as well; however, the limited database available has not allowed more than two frequency distribution curves to be described (Figure 3).

5. Concluding remarks

The EU SCOOP report is a complex collection of data from 10 European countries, focused on the levels of PCDDs, PCDFs, and dioxin-like PCBs in foodstuffs. For a statistical assessment, several issues represent an intrinsic difficulty. In particular, countries appear to have released their data in quite different ways (e.g., as results from pooled samples, as single results from single samples, as means of results from single samples), this introduces a remarkable lack of homogeneity into the data sets.

In the light of the above, the statistical appraisal presented here should be viewed as a pragmatic way to obtain basic estimates useful to characterise the occurrence of PCDDs, PCDFs, and dioxin-like PCBs in European foods. However, other statistical treatments could be adopted. The statistical figures obtained could possibly be used to support risk management strategies and/or exposure assessment exercises.

Owing to a possible interest in developing a specific risk management policy for fish farming, an *ad hoc* investigation should be carried out to collect additional experimental evidence and clarify whether a significant difference in dioxin and dioxin-like PCB levels exists between wild and farmed fish, a difference that may exist in the present data for farmed freshwater fish.

As stated under *Milk and milk products* (see Section 3), all “industrial”-type data present in the EU SCOOP report were eliminated by considering that milk from farms affected by industrial activity may be more contaminated than those away from industrial settings (as is apparently indicated by the available data). This observation might suggest a possible risk reduction measure for future pertinent initiatives.

The examples given within the context of risk management (see Section 4) clearly illustrate that, based on the available distribution curves, cut-off values for acceptance can be readily estimated for any selected percentile value.

6. References to Annex I

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Table 1. Statistical descriptors of dioxin concentrations in European foodstuffs since 1995 (original data from the EU SCOOP database, June 2000). Concentrations are expressed in pg I-TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg I-TEQ/g, whole food basis).

	UNWEIGHTED VALUES			*WEIGHTED VALUES*				
FOOD GROUP	**N1[a]**	**<X>**	**MED**	**N2[a]**	**<X>**	**SD**	**RANGE**	**COUNTRIES**
Cereals and cereal products	4	0.056	0.020	19	0.084	0.078	0.010–0.173	F, FIN, SV
—Cereals (fat <2%)	3	0.017	0.020	11	0.019	0.003	0.010–0.020	F, FIN
—Cereals (fat ≥2%)[b]	1	0.173	—	8	0.173	—	—	SV
Eggs	13	1.93	1.08	1300	1.26	0.640	0.460–7.32	DE, F, IT, NL, SV
Fats and oils[b]	1	0.770	—	8	0.770	—	—	SV
Fish and fish products	72	19.3	9.45	3288	20.6	28.7	0.125–225	More than six[c]
—Wild fish (marine, freshwater)[f]	56	22.0	10.4	3070	21.5	29.4	0.125–225	DE, F, IT, N, SV, UK
—Freshwater fish (culture)	14	10.9	9.19	203	10.2	5.39	2.33–27.9	DE, FIN, SV, UK
—Others[b]	2	—	—	15	—	—	2.73–4.36	UK
Fruit and vegetables	17	0.021	0.010	91	0.046	0.037	0.004–0.090	DE, F, FIN
Human milk	18	13.7	13.7	709	14.5	3.14	7.90–18.7	DE, F, SV
Meat and meat products	56	0.990	0.720	606	0.758	0.726	0.130–3.80	DE, F, IT, NL, SV
—Poultry	11	0.699	0.560	85	0.568	0.247	0.370–1.40	DE, IT, NL, SV
—Beef and veal	11	0.743	0.720	129	0.713	0.212	0.380–1.10	DE, IT, NL, SV
—Pork	22	0.853	0.400	227	0.342	0.421	0.130–3.80	DE, IT, NL, SV
—Game	3	1.63	1.94	39	1.85	0.300	0.970–1.97	DE, SV
—Others: liver	6	2.38	2.45	53	2.41	0.706	0.950–3.29	DE, F, IT, SV
mixed meat	3	0.553	0.630	73	0.570	0.159	0.270–0.760	F, SV
Milk and milk products[d]	57	0.882	0.620	2286	1.06	0.706	0.260–3.57	More than six[e]
—Milk as such[d]	40	0.965	0.610	1807	1.17	0.751	0.260–3.57	More than six[e]
—Others	17	0.685	0.640	479	0.618	0.085	0.300–1.50	DE, IT, NL, SV

[a] N1, actual number of analytical values appearing in the database; N2, correlated original sampling frequency.

[b] No statistics performed (N1 <3).

[c] DE, F, FIN, IT, N, SV, UK.

[c] Data associated with "industrial"-type specimens omitted (N1 = 7; N2 = 82).

[e] BE, DE, DK, F, FIN, IT, NL, SV, UK.

[f] Also includes some farmed salmon.

Table 1bis. Statistical descriptors of log-transformed dioxin concentrations in European foodstuffs since 1995 (original data from the EU SCOOP database, June 2000). Original concentrations are expressed in pg I-TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg I-TEQ/g, whole food basis).

	UNWEIGHTED VALUES			*WEIGHTED VALUES*				
FOOD GROUP	**N1[a]**	**μ**	**σ**	**N2[a]**	**μ**	**σ**	**RANGE**	**COUNTRIES**
Cereals and cereal products	4	-3.55	1.24	19	-3.04	1.14	-4.61– -1.76	F, FIN, SV
—Cereals (fat <2%)	3	-4.14	0.400	11	-3.98	0.209	-4.61– -3.91	F, FIN
—Cereals (fat ≥2%)[b]	1	-1.75	—	8	-1.75	—	—	SV
Eggs	13	0.362	0.743	1300	0.171	0.320	-0.777–1.99	DE, F, IT, NL, SV
Fats and oils[b]	1	-0.261	—	8	-0.261	—	—	SV
Fish and fish products	72	2.29	1.16	3288	2.28	1.27	-2.08–5.42	More than six[c]
—Wild fish (marine, freshwater)[f]	56	2.36	1.25	3070	2.29	1.23	-2.08–5.42	DE, F, IT, N, SV, UK
—Freshwater fish (culture)	14	2.18	0.702	203	2.18	0.559	0.847–3.33	DE, FIN, SV, UK
—Others[b]	2	—	—	15	—	—	1.00–1.47	UK
Fruit and vegetables	17	-4.22	0.817	91	-3.53	1.04	-5.52– -2.41	DE, F, FIN
Human milk	18	2.60	0.213	709	2.65	0.245	2.07–2.93	DE, F, SV
Meat and meat products	56	-0.367	0.856	606	-0.645	0.845	-2.04–1.34	DE, F, IT, NL, SV
—Poultry	11	-0.462	0.475	85	-0.645	0.389	-0.994–0.337	DE, IT, NL, SV
—Beef and veal	11	-0.350	0.350	129	-0.384	0.310	-0.968–0.095	DE, IT, NL, SV
—Pork	22	-0.712	1.02	227	-1.36	0.633	-2.04–1.34	DE, IT, NL, SV
—Game	3	0.437	0.405	39	0.595	0.214	-0.031–0.678	DE, SV
—Others: liver	6	0.797	0.446	53	0.819	0.391	-0.051–1.19	DE, F, IT, SV
mixed meat	3	-0.682	0.551	73	-0.616	0.360	-1.31–0.274	F, SV
Milk and milk products[d]	57	-0.352	0.637	2286	-0.125	0.570	-1.35–1.27	More than six[e]
—Milk as such[d]	40	-0.316	0.726	1807	-0.028	0.601	-1.35–1.27	More than six[e]
—Others	17	-0.437	0.348	479	-0.491	0.134	-1.20–0.406	DE, IT, NL, SV

[a] N1, actual number of analytical values appearing in the database; N2, correlated original sampling frequency.

[b] No statistics performed (N1 <3).

[c] DE, F, FIN, IT, N, SV, UK.

[d] Data associated with "industrial"-type specimens omitted (N1 = 7; N2 = 82).

[e] BE, DE, DK, F, FIN, IT, NL, SV, UK.

[f] Also includes some farmed salmon.

Table 2. Statistical descriptors of dioxin concentrations in European foodstuffs since 1995 (original data from the EU SCOOP database, June 2000). For statistical analysis, the original data sets and subsets were log-transformed and weighted. Concentrations are expressed in pg I-TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg I-TEQ/g, whole food basis).

FOOD GROUP	<X>[a]	<X> ± 2SD[a]	<X> ± 3SD[a]	CI(95%)[b]	CI(99%)[b]	COUNTRIES
Cereals and cereal products	0.048	0.005–0.464	0.002–1.45	0.006–0.386	0.001–2.21	F, FIN, SV
—Cereals (fat <2%)	0.019	0.012–0.029	0.010–0.035	0.010–0.035	0.004–0.081	F, FIN
—Cereals (fat ≥2%)[c]	0.173	—	—	—	—	SV
Eggs	1.19	0.626–2.25	0.455–3.10	0.970–1.45	0.895–1.57	DE, F, IT, NL, SV
Fats and oils[c]	0.770	—	—	—	—	SV
Fish and fish products	9.80	0.769–125	0.215–446	7.25–13.3	6.57–14.6	More than six[d]
—Wild fish (marine, freshwater)[g]	9.92	0.853–115	0.250–393	7.12–14.4	6.34–16.2	DE, F, IT, N, SV, UK
—Freshwater fish (culture)	8.84	0.525–16.8	0.221–40.0	6.32–12.4	5.54–14.1	DE, FIN, SV, UK
—Others[c]	2.73–4.36	—	—	—	—	UK
Fruit and vegetables	0.029	0.004–0.236	0.001–0.670	0.017–0.051	0.014–0.063	DE, F, FIN
Human milk	14.1	8.65–23.1	6.77–29.5	12.5–16.0	11.9–16.8	DE, F, SV
Meat and meat products	0.525	0.097–2.84	0.042–6.62	0.418–0.660	0.387–0.712	DE, F, IT, NL, SV
—Poultry	0.524	0.241–1.14	0.163–1.69	0.399–0.690	0.355–0.774	DE, IT, NL, SV
—Beef and veal	0.681	0.366–1.27	0.268–1.73	0.547–0.847	0.499–0.929	DE, IT, NL, SV
—Pork	0.258	0.073–0.914	0.039–1.72	0.193–0.343	0.174–0.381	DE, IT, NL, SV
—Game	1.81	1.18–2.78	0.953–3.45	0.945–3.48	0.403–8.15	DE, SV
—Others: liver	2.27	1.04–4.96	0.702–7.34	1.45–3.56	1.12–4.59	DE, F, IT, SV
mixed meat	0.540	0.263–1.11	0.184–1.59	0.181–1.62	0.043–6.76	F, SV
Milk and milk products[e]	0.882	0.275–2.84	0.153–5.08	0.757–1.03	0.720–1.08	More than six[f]
—Milk as such[e]	0.972	0.292–3.24	0.160–5.91	0.800–1.18	0.749–1.26	More than six[f]
—Others	0.612	0.469–0.800	0.410–0.914	0.570–0.657	0.555–0.675	DE, IT, NL, SV

[a] Weighting of <X> and SD estimates based on N2.

[b] Two-tail probability estimates; weighting of CI estimates based on N1.

[c] No statistics performed (N1 <3).

[d] DE, F, FIN, IT, N, SV, UK.

[e] Data associated with "industrial"-type specimens omitted (N1 = 7; N2 = 82).

[f] BE, DE, DK, F, FIN, IT, NL, SV, UK.

[g] Also includes some farmed salmon.

Table 3. Statistical descriptors of dioxin-like PCB concentrations in European foodstuffs since 1995 (original data from the EU SCOOP database, June 2000).[a] Concentrations are expressed in pg PCB-TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg PCB-TEQ/g, whole food basis).[b]

	UNWEIGHTED VALUES			*WEIGHTED VALUES*				
FOOD GROUP	**N1[c]**	**<X>**	**MED**	**N2[c]**	**<X>**	**SD**	**RANGE**	**COUNTRIES**
Cereals and cereal products[d, e]	1	0.110	—	8	0.110	—	—	SV
—Cereals (fat <2%)[e]	—	—	—	—	—	—	—	—
—Cereals (fat ≥2%)[d]	1	0.110	—	8	0.110	—	—	SV
Eggs[d]	2	—	—	132	—	—	0.440–1.45	NL, SV
Fats and oils[d]	1	0.420	—	8	0.420	—	—	SV
Fish and fish products	24	37.0	20.4	536	46.1	42.7	1.61–168	FIN, N, SV, UK
—Wild fish (marine, freshwater)[h]	17	43.2	23.7	418	53.5	45.5	1.61–168	N, SV, UK
—Freshwater fish (culture)	5	25.1	18.8	103	20.8	8.12	9.92–39.7	FIN, SV, UK
—Others[d]	2	—	—	15	—	—	11.8–16.6	UK
Fruit and vegetables[d, f]	2	—	—	2	—	—	0.030–0.120	FIN
Human milk[d]	1	9.90	—	63	9.90	—	—	SV
Meat and meat products	11	0.964	0.810	114	0.861	0.582	0.090–3.15	NL, SV
—Poultry[d]	2	—	—	19	—	—	0.590–0.700	NL, SV
—Beef and veal	3	0.970	0.970	40	0.923	0.120	0.860–1.08	NL, SV
—Pork[d]	2	—	—	27	—	—	0.090–0.810	NL, SV
—Game[d]	1	3.15	—	4	3.15	—	—	SV
—Others: liver[d]	2	—	—	16	—	—	0.270–1.65	SV
mixed meat[d]	1	0.430	—	8	0.430	—	—	SV
Milk and milk products[g]	8	0.670	0.540	173	1.31	0.645	0.230–1.80	FIN, NL, SV, UK
—Milk as such[g]	4	0.778	0.540	139	1.48	0.595	0.230–1.80	FIN, SV, UK
—Others	4	0.563	0.545	34	0.595	0.184	0.380–0.780	NL, SV

[a] "ICES7"-type PCB results omitted.

[b] TEQ contributions of dioxin-like PCBs estimated by using the PCB-TEF system proposed by Ahlborg *et al.* (1994).

[c] N1, actual number of analytical values appearing in the database; N2, correlated original sampling frequency.

[d] No statistics performed (N1 <3).

[e] Original data equal to 0.00 pg TE/g omitted (N1 = 2; N2 = 2).

[f] Original data equal to 0.00 pg TE/g omitted (N1 = 11; N2 = 11).

[g] Data associated with "industrial"-type specimens omitted (N1 = 9; N2 = 119).

[h] Also includes some farmed salmon.

Table 3bis. Statistical descriptors of log-transformed dioxin-like PCB concentrations in European foodstuffs since 1995 (original data from the EU SCOOP database, June 2000).[a] Original concentrations are expressed in pg PCB-TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg PCB-TEQ/g, whole food basis).[b]

FOOD GROUP	*UNWEIGHTED VALUES* N1[c]	μ	σ	*WEIGHTED VALUES* N2[c]	μ	σ	RANGE	COUNTRIES
Cereals and cereal products[d, e]	1	-2.21	—	8	-2.21	—	—	SV
—Cereals (fat <2%)[e]	—	—	—	—	—	—	—	—
—Cereals (fat ≥2%)[d]	1	-2.21	—	8	-2.21	—	—	SV
Eggs[d]	2	—	—	132	—	—	-0.821–0.372	NL, SV
Fats and oils[d]	1	-0.868	—	8	-0.868	—	—	SV
Fish and fish products	24	3.14	1.02	536	3.42	0.960	0.476–5.12	FIN, N, SV, UK
—Wild fish (marine, freshwater)[h]	17	3.21	1.17	418	3.56	1.03	0.476–5.12	N, SV, UK
—Freshwater fish (culture)	5	3.09	0.589	103	2.97	0.336	2.29–3.68	FIN, SV, UK
—Others[d]	2	—	—	15	—	—	2.47–2.81	UK
Fruit and vegetables[d, f]	2	—	—	2	—	—	-3.51–-2.12	FIN
Human milk[d]	1	2.29	—	63	2.29	—	—	SV
Meat and meat products	11	-0.374	0.935	114	-0.395	0.798	-2.41–1.15	NL, SV
—Poultry[d]	2	—	—	19	—	—	-0.528– -0.357	NL, SV
—Beef and veal	3	-0.113	0.187	40	-0.090	0.140	-0.329–0.010	NL, SV
—Pork[d]	2	—	—	27	—	—	-2.41– -0.211	NL, SV
—Game[d]	1	1.15	—	4	1.15	—	—	SV
—Others: liver[d]	2	—	—	16	—	—	-1.31–0.501	SV
mixed meat[d]	1	-0.844	—	8	-0.844	—	—	SV
Milk and milk products[g]	8	-0.580	0.612	173	0.069	0.724	-1.47–0.588	FIN, NL, SV, UK
—Milk as such[g]	4	-0.529	0.846	139	0.226	0.707	-1.47–0.588	FIN, SV, UK
—Others	4	-0.632	0.390	34	-0.572	0.338	-0.968– -0.249	NL, SV

[a] "ICES7"-type PCB results omitted.

[b] TEQ contributions of dioxin-like PCBs estimated by using the PCB-TEF system proposed by Ahlborg *et al.* (1994).

[c] N1, actual number of analytical values appearing in the database; N2, correlated original sampling frequency.

[d] No statistics performed (N1 <3).

[e] Original data equal to 0.00 pg TE/g omitted (N1 = 2; N2 = 2).

[f] Original data equal to 0.00 pg TE/g omitted (N1 = 11; N2 = 11).

[g] Data associated with "industrial"-type specimens omitted (N1 = 9; N2 = 119).

[h] Also includes some farmed salmon.

Table 4. Statistical descriptors of dioxin-like PCB concentrations in European foodstuffs since 1995 (original data from the EU SCOOP database, June 2000).[a] For statistical analysis, the original data sets and subsets were log-transformed and weighted. Concentrations are expressed in pg PCB-TEQ/g, lipid basis, except for cereals, cereal products, fruit, and vegetables (pg PCB-TEQ/g, whole food basis).[b]

FOOD GROUP	<X>[c]	<X> ± 2SD[c]	<X> ± 3SD[c]	CI(95%)[d]	CI(99%)[d]	COUNTRIES
Cereals and cereal products[e]	0.110	—	—	—	—	SV
—Cereals (fat <2%)[e]	—	—	—	—	—	—
—Cereals (fat ≥2%)[e]	0.110	—	—	—	—	SV
Eggs[e]	0.440–1.45	—	—	—	—	NL, SV
Fats and oils[e]	0.420	—	—	—	—	SV
Fish and fish products	30.7	4.50–209	1.72–546	20.3–46.4	17.5–53.8	FIN, N, SV, UK
—Wild fish (marine, freshwater)[g]	35.3	4.51–276	1.61–773	20.5–60.9	16.7–74.9	N, SV, UK
—Freshwater fish (culture)	19.6	9.99–38.3	7.14–53.6	12.3–31.2	9.03–42.4	FIN, SV, UK
—Others[e]	11.8–16.6	—	—	—	—	UK
Fruit and vegetables[e]	0.030–0.120	—	—	—	—	FIN
Human milk[e]	9.90	—	—	—	—	SV
Meat and meat products	0.674	0.137–3.32	0.062–7.37	0.384–1.18	0.303–1.50	NL, SV
—Poultry[e]	0.590–0.700	—	—	—	—	NL, SV
—Beef and veal	0.914	0.691–1.21	0.600–1.39	0.597–1.40	0.342–2.44	NL, SV
—Pork[e]	0.090–0.810	—	—	—	—	NL, SV
—Game[e]	3.15	—	—	—	—	SV
—Others: liver[e]	0.270–1.65	—	—	—	—	SV
mixed meat[e]	0.430	—	—	—	—	SV
Milk and milk products[f]	1.07	0.252–4.56	0.122–9.40	0.561–2.05	0.411–2.79	FIN, NL, SV, UK
—Milk as such[f]	1.25	0.305–5.15	0.150–10.5	0.342–4.59	0.115–13.6	FIN, SV, UK
—Others	0.564	0.287–1.11	0.205–1.55	0.303–1.05	0.181–1.76	NL, SV

[a] "ICES7"-type PCB results omitted.

[b] TEQ contributions of dioxin-like PCBs estimated by using the PCB-TEF system proposed by Ahlborg *et al.* (1994).

[c] Weighting of <X> and SD estimates based on N2.

[d] Two-tail probability estimates; weighting of CI estimates based on N1.

[e] No statistics performed (N1 <3).

[f] Data associated with "industrial"-type specimens omitted (N1 = 9; N2 = 119).

[g] Also includes some farmed salmon.

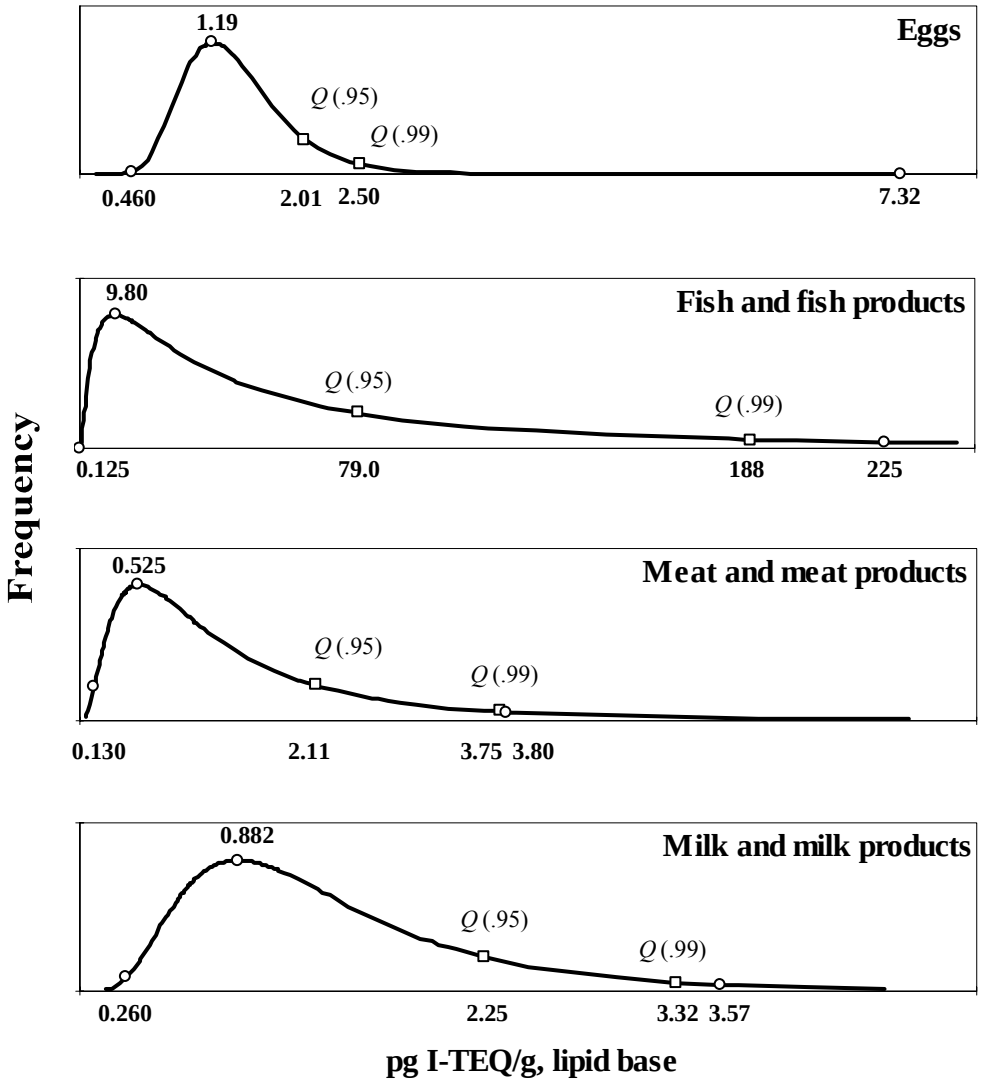

Figure 1. Frequency distribution curves of PCDD and PCDF concentrations in various foods of animal origin. The concentration figures identified by (o) are, from left to right, X_{min}, <X>, and X_{max}. The 95th and 99th percentiles are identified from left to right by (□). The range covered by the fish distribution is between one and two orders of magnitude greater than those of the other foods.

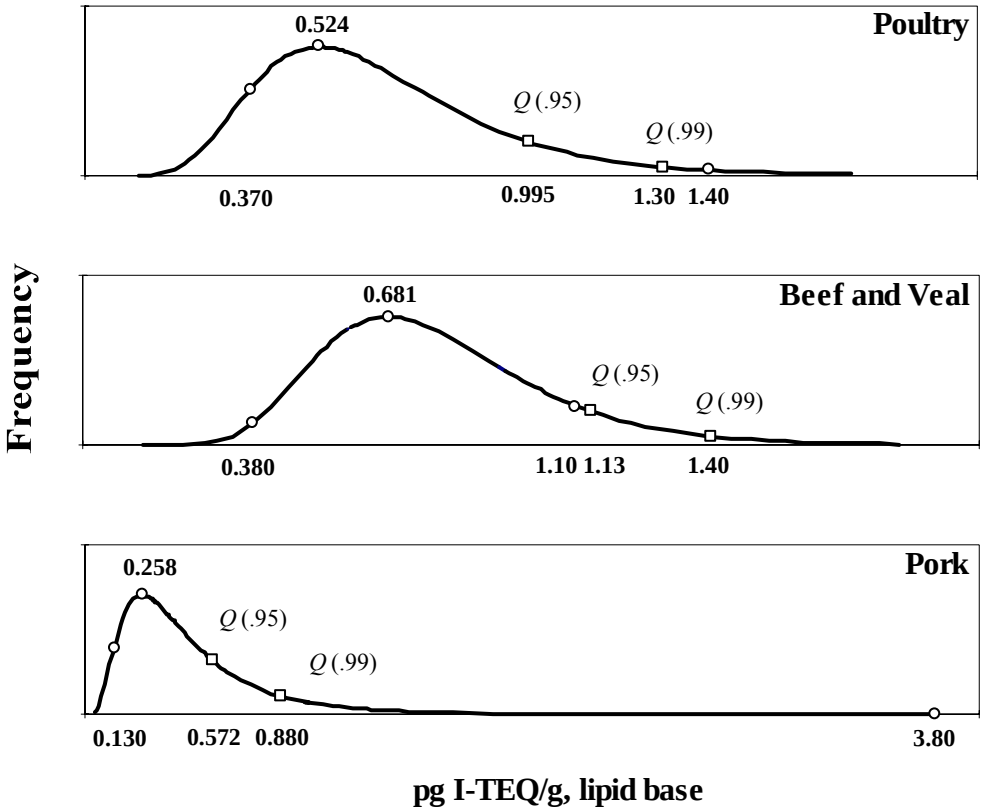

Figure 2. Frequency distribution curves of PCDD and PCDF concentrations in three food subgroups of animal origin. The concentration figures identified by (o) are, from left to right, X_{min}, <X>, and X_{max}. The 95th and 99th percentiles are identified from left to right by (□).

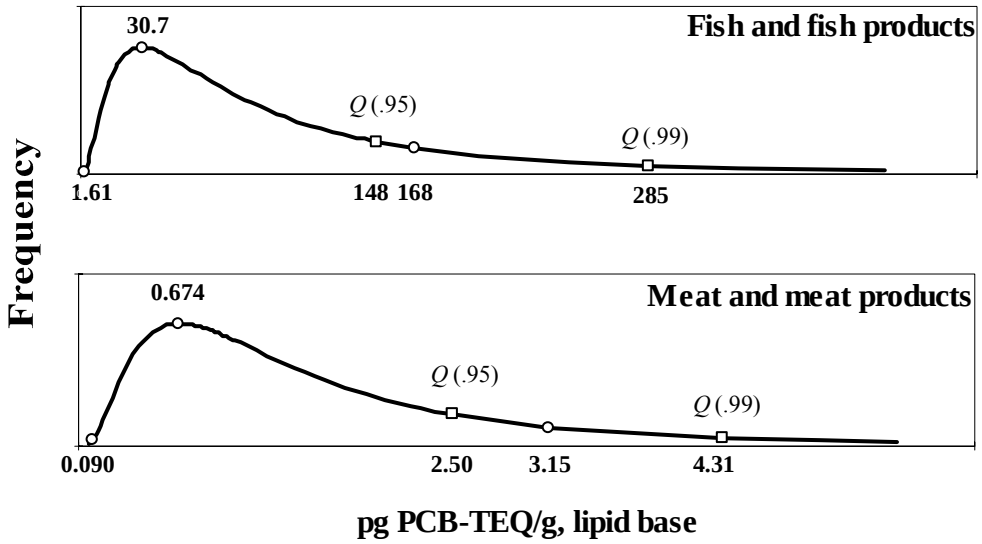

Figure 3. Frequency distribution curves of dioxin-like PCB concentrations in foods of animal origin. The concentration figures identified by (o) are, from left to right, X_{min}, <X>, and X_{max}. The 95th and 99th percentiles are identified from left to right by (□). The range covered by the fish distribution is between one and two orders of magnitude greater than that of the meat food group.

ANNEX II

SUMMARY OF HEALTH EFFECTS OF DIOXINS

Summary of some of the draft background monographs originally prepared for the WHO Consultation on the re-evaluation of dioxins and dioxin-like compounds, Geneva, 1998 (WHO, 1998). It therefore does not constitute a new original and formal review, but serves to inform the Committee members. The authors of the WHO background papers are acknowledged at appropriate places. The full report of the WHO Consultation is now available (WHO, 2000).

Table of contents

1. Toxicological effects of dioxins. Experimental studies

A number of reviews are available on the toxicological properties of dioxins and dioxin-like PCBs (Birnbaum, 1994; Pohjanvirta and Tuomisto, 1994; Gasiewicz, 1997; IARC, 1997; ASTDR, 1997; Giesy and Kannan, 1998). Many of the toxic effects of dioxins are "high-dose" effects. They are briefly summarised in this assessment, which focus on studies and issues related to the establishment of a tolerable intake for dioxins and dioxin-like PCBs. Special emphasis is paid to the critical effects identified by a WHO Consultation in 1998 in its re-evaluation of the TDI (WHO, 1998 and 2000).

1.1 Absorption, distribution, biotransformation and excretion (based on van Birgelen and van den Berg, 1998)

Three factors govern the toxicokinetics of dioxins and dioxin-like compounds: the lipophilicity of the compound, binding to cytochrome P4501A2 (in the liver), and the rate of metabolism.

The lipophilicity controls the absorption rate, tissue distribution and passive elimination. Molecular size and solubility are the rate limiting factors for the absorption from the GI tract (van den Berg *et al.*, 1994). Dioxins having 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 (Poiger and Schlatter, 1986; van den Berg *et al.*, 1994; IARC, 1997). 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. There are experimental studies indicating that PCDFs are transferred to the foetus to a lesser extent than PCDDs (IARC, 1997; Weber and Birnbaum, 1985).

Binding to cytochrome P4501A2 results in sequestration of dioxin-like compounds in the liver. CYP1A2 is induced by dioxin-like compounds (DeVito *et al.*, 1998). The increase in CYP1A2 results in a redistribution of dioxin-like compounds from the adipose tissue to the liver. In rodents, a marked redistribution to the liver has been found after exposure to dose levels of dioxin-like compounds which result in hepatic CYP1A2 induction (DeVito *et al.*,

1995a). In humans, no clear hepatic sequestration has been observed although there are indications that limited hepatic sequestration occurs, mainly for higher chlorinated PCDDs and PCDFs (Thoma *et al.*, 1990; Kreuzer *et al.*, 1997). However, estimates by Liem and Theelen (1997) indicate that only 1% of the total human body burden is located in the liver.

Metabolism of dioxin-like compounds leads to detoxification. The major determinant for metabolism of PCDDs and PCDFs (and PCBs) is the presence of two adjacent, unsubstituted carbon atoms on the lateral positions (van den Berg *et al.*, 1994). The Ah receptor-dependent cytochrome P4501A1 has been associated with the oxidative metabolism of certain PCDD, PCDF, and PCB congeners (Tai *et al.*, 1993; Murk *et al.*, 1994).

In rats, the half-life of 2,3,7,8-TCDD ranged from 12 to 31 days (van den Berg *et al.*, 1994) and the half-life of 2,3,7,8-TCDD in humans has been reported to range from 5 to 11 years (Olson, 1994; Flesch-Janys *et al.*, 1996; Michalek *et al.*, 1996). The half-life has been shown to increase with age, probably due to increase in adipose tissue and decrease in metabolism (Flesch-Janys *et al.*, 1996). Breast-fed infants had higher levels of PCDDs and PCDFs than non-breast-fed infants in both adipose tissue and liver. However, even after 19 weeks of breast feeding these tissue levels never exceeded those in adults (Thoma *et al.*, 1990; Kreuzer *et al.*, 1997). In addition, no increase in the concentration of PCDDs, PCDFs, planar PCBs, and total TEQs in adipose tissue due to breast-feeding was predicted over an human lifetime (Kreuzer *et al.*, 1997; Liem and Theelen, 1997).

Evidence for transplacental transfer of dioxins in humans has been obtained from analysis of foetal tissues. Human foetuses of 8-14 weeks gestational age contained approximately 30% of the I-TEQ of human milk (5.3 ng/kg lipid vs. 16.7 ng/kg lipid) (Schecter *et al.*, 1996a, 1996b). Cord blood samples obtained at birth are representative of foetal circulation and therefore also of *in utero* exposure to contaminants. When expressed on a lipid basis, concentrations of PCBs in cord blood samples were comparable to the maternal plasma values (Koopman-Esseboom *et al.*, 1994a). Limited information suggests that a similar situation exists for 2,3,7,8-TCDD (Needham *et al.*, 1991).

Quantitatively, human newborns are exposed to larger amounts of organic contaminants through breast-feeding than in any other stage of development or life. It has been estimated that on average the maternal body burden of PCDDs/DFs can decrease between 20-30% during lactation (Beck *et al.*, 1994). Newborns who breast feed can have estimated I-TEQ intakes up to 50-fold greater than infants maintained on formula. Based on current average concentrations of 10-30 ng/kg lipid I-TEQ in breast milk from industrialised countries, an infant who breast-feeds will be exposed to approximately 50-100 pg I-TEQ/kg bw/day. This is in comparison to an estimated intake by formula-fed infants or the general population of generally less than 2 pg I-TEQ/kg bw/day (Abraham *et al.*, 1996).

1.2. Mode of action (based on Poellinger, 1998)

Most, if not all, of the biochemical and toxicological effects of the dioxins are thought to depend on binding to a specific receptor protein in the cells, the Ah receptor (IARC, 1997; Gasiewitz, 1997). The Ah receptor is a soluble, intracellular protein, which functions as a ligand-activated transcription factor that mediates the pleiotropic biological responses to dioxins (Hankinson, 1995; Poellinger, 1995; Schmidt and Bradfield, 1996; Sogawa and Fujii-Kuriyama, 1997; Whitlock *et al.*, 1997; IARC, 1997). Studies using Ah receptor-deficient mice have demonstrated the receptor's role in mediating adaptive metabolic responses to

polycyclic aromatic hydrocarbons and dioxins, acute toxic effects in thymus and liver, and teratogenic effects (Fernandez-Salguero *et al.*, 1995; Schmidt *et al.*, 1996; Mimura *et al.*, 1997).

The receptor binds 2,3,7,8-TCDD and its planar congeners in a saturable manner and with high affinity and is expressed in virtually every tissue of adult rodents and humans. In humans a single dioxin receptor gene has been identified on chromosome 7 showing polymorphism (Fujii-Kuriyama *et al.*, 1995). In the absence of ligand the Ah receptor is present in the cytosolic compartment of the cell in a non-DNA binding form associated with the molecular chaperone hsp90 (Wilhelmsson *et al.*, 1990). Upon exposure to a ligand, e.g. 2,3,7,8-TCDD, the receptor is translocated into the cell nucleus (Ikuta *et al.*, 1998). DNA binding activity is acquired following release of hsp90 and dimerization with the Arnt protein. The nuclear ligand-activated Ah receptor-Arnt complex specifically recognises xenobiotic response elements (XREs) and activates transcription of a battery of dioxin-inducible genes (Hankinson, 1995; Poellinger, 1995; Schmidt and Bradfield, 1996; Sogawa and Fujii-Kuriyama, 1997; Whitlock *et al.*, 1997). Primary target genes identified so far are a number of genes encoding drug metabolising enzymes such as cytochrome P4501A1 and gluthathione-*S*-transferase Ya (Hankinson, 1995; Whitlock *et al.*, 1997).

The physiological function of the dioxin receptor in mammals is not known. The significant conservation of the protein in all vertebrate groups (Hahn *et al.*, 1997), insects (Duncan *et al.*, 1998), and worms (Powell-Coffman *et al.*, 1998) suggests that it has a fundamental role in cellular physiology. Studies using receptor-deficient mice have documented a spectrum of pathological lesions (Fernandez-Salguero *et al.*, 1995, 1996, 1997; Androla *et al.*, 1997) and indicated a role of the receptor in the normal growth and development of the liver and the immune system (Fernandez-Salguero *et al.*, 1995; Gonzalez, 1996; Schmidt *et al.*, 1996).

1.3. Biochemical effects (based on Birnbaum and Tuomisto, 1998)

Induction of certain drug metabolising enzymes, especially those of the CYP1A family, is a sensitive response to dioxin exposure. CYP1A1 expression is extremely low in most tissues without exposure to dioxin or other Ah receptor ligands. In contrast, there is constitutive hepatic expression of CYP1A2 (Diliberto *et al.*, 1997). In the rat, Vanden Heuvel *et al.* (1994) demonstrated significant induction of CYP1A1 mRNA following a single dose of 1 ng/kg bw but not after 0.1 ng/kg bw. Induction of the enzymatic activity of CYP1A1, e.g. ethoxyresorufin-O-deethylase (EROD) activity, parallels that of the mRNA and has been demonstrated in subchronic studies in rats and mice at dose levels 0.1-0.3 ng 2,3,7,8-TCDD/kg bw/day (Vogel *et al.*, 1997; Kohn *et al.*, 1993; DeVito *et al.*, 1994; Tritscher *et al.*, 1992; van Birgelen *et al.*, 1995; Li and Rozman, 1995; DeVito *et al.*, 1997b; Diliberto *et al.*, 1998). The NOEL was approximately 0.03 ng/kg bw/day (Vogel *et al.*, 1997). Induction of CYP1A2 mRNA and related enzyme activities has been demonstrated at similar doses to that of CYP1A1 (Vogel *et al.*, 1997; DeVito *et al.*, 1994, 1997b). Several studies have indicated that induction of CYP1A2 may be an even more sensitive response than induction of hepatic CYP1A1 (Vogel *et al.*, 1997; Narisimhan *et al.*, 1994; DeVito *et al.*, 1993, 1994).

Activation of the expression of other drug metabolising enzymes, such as CYP1B1, aldehyde dehydrogenase, glutathione S-transferase (GSTYa), glucuronosyl transferase (UGT1A1), and DT diaphorase (also known as NAD(P)H:quinone oxidoreductase), appear to be less sensitive (Abel *et al.*, 1996; Vanden Heuvel *et al.*, 1994) and show a different time course of induction (Santostefano *et al.*, 1997).

2,3,7,8-TCDD exposure has been shown to affect several *growth factor systems*. Many of the effects of dioxins resemble those seen with either hyper- or hypo-vitaminosis A (Abbott *et al.*, 1989). Single or chronic exposure to dioxins results in a progressive and dose-related decline in the major hepatic storage form of vitamin A, retinyl palmitate (Jurek *et al.*, 1990, DeVito *et al.*, 1997a) due to enhanced metabolism. The decrease in hepatic storage is accompanied by an increase in retinoids in serum and kidney. 2,3,7,8-TCDD exposure also alters the expression and signalling of the mitogenic growth factors, EGF and TGF, in a time and tissue-dependent manner. Studies have shown EGF to be down-regulated in the placenta (Ryan *et al.*, 1989), liver (Sewall *et al.*, 1995) and the developing ureter (Bryant *et al.*, 1997) and elevated in the developing palate (Abbott and Birnbaum, 1989) and urinary tract (Abbott and Birnbaum, 1990a). Down regulation of the EGF receptor in the liver occurs at the same dose as induction of CYP1A1 and CYP1A2 (Kohn *et al.*, 1993). Several members of the inhibitory growth factor family TGF beta are also affected by 2,3,7,8-TCDD exposure. Increases in both TGF beta 1 and 2 have been seen in the developing palate following 2,3,7,8-TCDD exposure (Abbott and Birnbaum, 1990b; Hebert *et al.*, 1990b). Induction of tyrosine kinase activity in a number of tissues also appears to be a sensitive response. Subchronic exposure to mice demonstrated significant induction of tyrosine phosphorylation of cell cycle proteins at 1.5 ng/kg bw/day (DeVito *et al.*, 1994).

Dioxin exposure can produce a number of effects on the *endocrine system*. Multiple hormone systems have been affected in experimental animals. Dioxin may perturb levels of hormones, the number of hormone receptors, and the serum transport of hormones (Gasiewicz, 1997). Thyroxine concentrations are often found to be decreased due to enhanced glucuronidation and elimination (Schuur *et al.*, 1997). A decrease in total T4 levels may be associated with an increase in TSH levels, due to a compensatory feedback loop *via* the hypothalamus and pituitary (Sewall *et al.*, 1995). 2,3,7,8-TCDD exposure induced a decrease in blood insulin (Gorski and Rozman, 1987) and glucose (Birnbaum *et al.*, 1990), as well as a reduction in glucose transporting activities in adipose tissue and the pancreas (Enan *et al.*, 1992). In contrast, 2,3,7,8-TCDD exposure leads to an increase in serum gastrin levels (Theobald *et al.*, 1991). 2,3,7,8-TCDD exposure can disrupt the normal feedback mechanisms of the pituitary between plasma levels of testosterone, dihydrotestosterone (DHT) and oestradiol and LH secretion (Bookstaff *et al.*, 1990). Effects on the adrenals involve increases in ACTH resulting in altered levels of circulating glucocorticoids (Bestervelt *et al.*, 1993). The effects on hormone receptors appear to be tissue specific. A decrease in the number of glucocorticoid receptors has been observed in the liver of rats and mice (Lin *et al.*, 1991; Ryan *et al.*, 1989), in the placenta of mice (Ryan *et al.*, 1989), and in the muscle of rats (Max and Silbergeld, 1987). In the developing palate, 2,3,7,8-TCDD up-regulates the number of glucocorticoid receptors (Abbott *et al.*, 1994). Dioxins can also alter the metabolism of both estrogens and androgens and decrease the number of oestrogen receptors in uterine and liver tissue (Safe *et al.*, 1991). Many of these effects occur at relatively high doses.

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 have been shown in the liver as well as extrahepatic tissues following exposure to high doses of 2,3,7,8-TCDD (Stohs, 1990). Induction of CYP1A isoforms by 2,3,7,8-TCDD is associated with oxidative DNA damage (Park *et al.*, 1996). Altered metabolism of endogenous molecules such as oestradiol can lead to the formation of quinones and redox cycling. This has been hypothesised to play a role in the enhanced sensitivity of female rats to 2,3,7,8-TCDD-induced liver tumours (Tritscher *et al.*, 1996). Low-dose (0.45 ng/kg/day) chronic

exposure to 2,3,7,8-TCDD can lead to oxidative damage in the brain of exposed mice (Hassoun *et al.*, 1998).

1.4. Toxicological effects in adult animals (based on Birnbaum and Tuomisto, 1998)

Most animal studies have used the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), the most toxic congener.

Lethality is the effect that varies most among species, i.e. the LD50 varies from 1 μg/kg bw in the guinea pig to >1000 μg/kg bw in the hamster. Rats, mice, dogs, cattle, horses, and non-human primates are intermediate in sensitivity to dioxin-induced lethality. Death follows a characteristic wasting syndrome (after 1-2 weeks in rabbits and guinea pigs, 2-3 weeks in rats, 3-4 weeks in mice and 6-7 weeks in monkeys) in which animals mobilise their body fat and muscle mass. Wasting results mainly, if not exclusively, from hypophagia, and the depletion of energy stores seems to be an important determinant of the time-to-death. The effect is largely independent of dose, as long as it exceeds a lethal dose (e.g., animals do not die faster because the dose is 10 or 100 times a lethal dose). Atrophy of the thymus is one of the most characteristic effects 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 than that of the thymus and does not occur in all species (e.g. rats). The adult testis and ovary have similar sensitivity to that of the spleen (Viluksela *et al.*, 1997).

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. Proliferative responses are primarily a high-dose effect (Fox *et al.*, 1993). Accumulation of porphyrins also requires relatively high doses of 2,3,7,8-TCDD and is potentiated by iron, which indicate that oxidative stress may be involved in the hepatic toxicity (Smith *et al.*, 1998).

The *cardiovascular system* appears to be a very sensitive target for 2,3,7,8-TCDD in chickens, resulting in oedema. In mammalian species, oedema is sometimes seen, but usually requires high dose levels, as do alterations in cardiac function and morphology.

The *gastrointestinal tract*, i.e. the stomach, undergoes hyperplasia in response to toxic doses of 2,3,7,8-TCDD in several species, such as non-human primates. Monkeys also respond to 2,3,7,8-TCDD with squamous metaplasia of the meibomian glands on the *eyelids*, and the ceruminous glands lining the *ear canal*.

Adult animals exposed to relatively high 2,3,7,8-TCDD doses exhibit behavioural signs indicative of effects on the *central nervous system* (Unkila *et al.*, 1995; Tuomisto *et al.*, 1995; Gasiewicz, 1997). Some effects on nerve conduction velocity have been reported which may be associated with a progressive neuropathy (Grehl *et al.*, 1993).

The *immune system* is a target for 2,3,7,8-TCDD toxicity in multiple animal species and 2,3,7,8-TCDD has caused suppression of both cell-mediated and humoral immunity. These effects are seen at doses which are also associated with thymic atrophy and other overt signs of toxicity. However, doses of 100 ng/kg bw are associated with multiple effects on various immune parameters. The classic model for immunotoxicity by 2,3,7,8-TCDD is suppression

of the primary antibody response to sheep red blood cells. In responsive mice, the lowest ED50 for this response after single dose exposure has been observed at 91 ng/kg bw (Narasimhan *et al.*, 1994). After subchronic exposure a significant decrease in the PFC response was reported at 1.5 ng/kg bw/day (Smialowicz *et al.*, 1997). Suppression of the PFC response does not occur in rats, where toxic doses of 2,3,7,8-TCDD in fact lead to an enhancement (Smialowicz *et al.*, 1994).

In mice, a decrease in thymic cellularity, accompanied by a uniform depletion of all four subsets of T cells has been demonstrated (Rhile *et al.*, 1996). Earlier studies (Clark *et al.*, 1981), which indicated that the cytotoxic T cell response occurred following four weekly doses of 4 ng/kg, could not be replicated by Hanson and Smialowicz (1994). No effect was observed on this response at 3 μg/kg given four times over a month. In rats this response was less sensitive than thymic atrophy (Rice *et al.*, 1995). However, suppression of the function of several T cell subsets was observed after dietary exposure of rats to approximately 10 ng/kg bw/day (Badesha *et al.*, 1995). Subtle changes in immunophenotypes were observed following chronic exposure of mice to approximately 30 ng/kg bw/day (Oughton *et al.*, 1995).

The most sensitive immunological effect reported in rodents is enhanced mortality due to influenza. Treatment of mice with 10 ng/kg bw one week prior to challenge with influenza virus resulted in increased mortality (Burleson *et al.*, 1996). No effect was seen following 1 or 5 ng/kg of 2,3,7,8-TCDD. Increased expression of the cytokine, IL-1 beta was seen in mice after daily doses of 0.3 ng/kg bw/day, which resulted in a similar body burden as acute exposure to 10 ng/kg bw (Vogel *et al.*, 1997).

Rhesus monkeys fed 25 parts per trillion (ppt) 2,3,7,8-TCDD in their diets (approximately 0.8 ng/kg bw/day) for four years (Hong *et al.*, 1989) had an increase in the number of cytotoxic/suppressor T cells and a decreased number of helper/inducer cells. This led to a lower CD4/CD8 ratio than in the control animals. However, there was no clinical evidence of any immune defect in the animals. Using marmoset monkeys, Neubert and coworkers (1990) reported that acute exposure to 2,3,7,8-TCDD resulted in a shift in the CD4/CD8 ratio, although the total number of T cells was not changed. The helper cell most affected was the "memory" subset of CD4 cells, with a significant decrease at 10 ng/kg bw. No effect was observed at 3 ng/kg bw. However, repeated exposure to a dose of 0.3 ng/kg bw/week led to an increase in this population of cells (Neubert *et al.*, 1992). When animals were administered 1.5 ng/kg bw/week (approximately 0.2 ng/kg bw/day), a decrease in the "memory" cells was observed again. These contradictory responses raise concerns about using these immune responses for extrapolation to very low doses. No functional deficits were associated with the altered lymphocyte subset profiles (Neubert *et al.*, 1993).

High doses of 2,3,7,8-TCDD have been shown to impair both the adult male and female *reproductive system*. Subchronic exposure of male rats to 1 μg/kg bw/day resulted in decreased spermatogenesis (Kociba *et al.*, 1976) and a single dose of 15 μg/kg bw produced a decrease in the levels of circulating androgens (Moore *et al.*, 1985) and effects on the Leydig cells (Johnson *et al.*, 1994). Ovarian dysfunction occurs in several species at high doses of 2,3,7,8-TCDD, for example following 10 μg/kg bw to female rats (Li *et al.*, 1995). 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 NOAEL/LOAEL of 1 ng/kg bw/day for reproductive impairment in the F1 and F2 generations (Murray *et al.*, 1979; Nisbet and Paxton 1982). Effects were clearly observed at a daily dose of 10 ng/kg bw/day. Exposure of rhesus monkeys to 25 ppt in the diet (approximately 0.8

ng/kg bw/day) led to foetal loss due to spontaneous abortions in seven of eight pregnant animals (Bowman *et al.*, 1989; Schantz and Bowman, 1989). A dose-related increase in the incidence and severity of *endometriosis* was observed when these female rhesus monkeys and an additional group administered 2,3,7,8-TCDD at a level of 0,5 ppt 2,3,7,8-TCDD in their diet for up to four years were held for as long as ten additional years without treatment (Rier *et al.*, 1993). In rats and mice, growth of surgically induced endometriotic cysts has also been promoted by exposure to 2,3,7,8-TCDD (and 2,3,4,7,8-PnCDF), although at much higher doses than in the monkey. PCB 126 produced ovarian toxicity, while the non-dioxin-like compounds, PCB 153 and 1,3,6,8-TCDD had no effects on endometriosis (Cummings *et al.*, 1996; Johnson *et al.*, 1997).

1.5. Toxicological effects following prenatal (*in utero*) exposure to animals (based on Birnbaum and Tuomisto, 1998)

Adverse *developmental effects* e.g. toxicity, growth retardation, thymic and splenic atrophy, haemorrhage, and oedemas have been observed in foetuses and neonates of many species. Frank structural malformations, i.e. hydronephrosis and cleft palate, have been induced by 2,3,7,8-TCDD in mice (Birnbaum, 1991). High prenatal doses of 2,3,7,8-TCDD lead to accelerated tooth eruption in mice (Madhukar *et al.*, 1984) and impaired dentin and enamel formation in continuously growing rat incisors (Alaluusua *et al.*, 1993).

Prenatal exposure has been shown to result in permanent adverse effects on the *reproductive system* of both male and female offspring in rats (Mably *et al.*, 1992a, b, c; Gray *et al.*, 1995; Gray and Ostby, 1995; Gray *et al.*, 1997a, b). Male pups demonstrate delayed puberty, altered mating behaviour, and decreased sperm counts. Female pups show genital malformation consisting of vaginal threads and cleft phallus. Premature reproductive senescence in female pups has also been observed (Gray and Ostby, 1995). These effects have all been shown to be caused by *in utero* exposure (Bjerke and Peterson, 1994; Gray *et al.*, 1997a, b). Similar effects have been seen in hamsters (Gray *et al.*, 1995) while mice appear less sensitive (Theobald and Peterson, 1997). In rats, the decrease in male sperm count has been observed following a single gavage exposure on gestation day (GD) 15 at doses as low as 50 and 64 ng/kg bw (Gray *et al.*, 1997a; Mably *et al.*, 1992c). Premature eye opening was also seen at 50 ng/kg bw (Gray *et al.*, 1997a, b). In female offspring, the increase in the incidence of the vaginal thread was statistically significant at 200 ng/kg bw (Gray *et al.*, 1997b). In studies that showed the same incidence of adverse effects in the offspring of pregnant rats after exposure to 0.2 μg/kg bw on GD15 as compared to 1 μg/kg bw on GD8, similar foetal tissue 2,3,7,8-TCDD concentrations were measured on GD16 (13.2 pg/g foetus after exposure to 0.2 μg/kg on GD15 and 15.3 pg/g foetus after 1 μg/kg/day on GD8) (Hurst *et al.*, 1996, 1997, 1998a, 1998b). This supports the concept that the tissue concentration at the critical window of sensitivity is the key dose metric.

Neurobehavioural effects of prenatal 2,3,7,8-TCDD exposure have been associated with permanent changes. Exposure of pregnant rats to 0.3 μg/kg on GD19 led to hearing deficits in the offspring. This effect could be at least partially blocked with exogenous thyroxine (Goldey *et al.*, 1995, 1996). A similar exposure regimen resulted in changes in locomotor activity and rearing behavior (Thiel *et al.*, 1994). Exposure of the dam to 1 μg/kg on GD15 in rats or 2 μg/kg on GD12 in hamsters resulted in a permanent depression of the core body temperature (Gordon *et al.*, 1995, 1996). The offspring of rhesus monkey dams chronically fed 5 ppt 2,3,7,8-TCDD in the diet (approximately 0.15 ng/kg bw/day for a total of four years) had deficits in object learning; but improvements in spatial learning (Schantz and Bowman,

1989). These effects have also been seen in rats exposed on GD10-16 to 0.1 μg/kg bw/day (Schantz *et al.*, 1996). The chronic exposure of the monkey dams led to a maternal body burden of approximately 42 ng/kg bw (DeVito *et al.*, 1995b).

Subtle effects on the *immune system*, such as changes in cell surface markers, have been observed after prenatal doses of 2,3,7,8-TCDD lower than those inducing atrophy of the thymus in both mice (Holladay *et al.*, 1991) and rats (Gehrs and Smialowicz, 1997a, b; Ross *et al.*, 1997). A single gestational exposure of pregnant rats to 0.1 μg/kg bw resulted in a permanent suppression of delayed-type hypersensitivity in the offspring (Gehrs and Smialowicz, 1997b, 1998). The window of sensitivity for this effect appeared to be in the first postnatal days, as determined by cross-fostering studies.

1.6. Carcinogenicity studies in experimental animals (based on Dragan and Schrenk, 1998)

The results of a two-year chronic bioassay performed by Kociba *et al.* (1978) has for many years formed the primary basis for the evaluation of 2,3,7,8-TCDD (IARC, 1987). Groups of male and female Sprague-Dawley rats were administered 2,3,7,8-TCDD in their diets at dose levels of 0, 1, 10, and 100 ng 2,3,7,8-TCDD/kg bw/day. The female rat liver was the primary target site. Hepatic hyperplastic nodules were seen at an incidence of 8/86, 3/50, 18/50, and 23/50 for control, 1, 10, and 100 ng/kg bw/day, respectively. Similarly, the incidence of hepatocellular carcinomas in female rats was 1/86, 0/50, 2/50, and 11/50. A re-evaluation of the lesions in the female rat liver of 2,3,7,8-TCDD-treated animals found a similar trend in the occurrence of hepatic tumours (Goodman and Sauer, 1992), but at a lower incidence. Toxic hepatitis was observed in the 2,3,7,8-TCDD-treated females with liver tumours. Hepatic tumours were not observed in the male rats. In addition, lung tumours were also observed in the female rats, and the incidence was statistically increased at 100 ng/kg bw/day. The NOAEL for hepatic neoplasms was 1 ng/kg bw/day, based on the combined incidence of adenomas and carcinomas (Paustenbach *et al.*, 1990; Shu *et al.*, 1987). The correlation of overt hepatotoxicity with tumour formation, coupled with the low incidence of hepatocellular carcinoma formation and their well-differentiated nature, suggested that 2,3,7,8-TCDD is a weak carcinogen for the female rat liver (Goodman and Sauer, 1992). The body burden of 2,3,7,8-TCDD at the no effect level of 1 ng/kg bw/day was 60 ng 2,3,7,8-TCDD/kg bw.

The carcinogenicity of 2,3,7,8-TCDD has also been assessed in Osborne Mendel rats and B6C3F1 mice (NTP, 1982). Male and female rats and male mice were administered 10, 50 and 500 ng 2,3,7,8-TCDD /kg bw/week by gavage. Female mice were administered 40, 200 and 2000 ng 2,3,7,8-TCDD/kg bw/week. Increased incidences were seen in follicular cell thyroid adenomas in male rats and hepatocellular carcinomas and adrenal cortical adenomas/carcinomas in female rats at the highest dose levels. Neoplastic nodules were also observed in female rats at the highest dose. The thyroid follicular cell adenomas observed in the male rats were increased in a dose dependent manner with a trend toward an increase at the lowest dose. At the highest dose, an increased incidence of hepatocellular carcinomas was also observed in male mice. Subcutaneous fibrosarcomas as well as a marginal increase in thyroid follicular adenomas were observed in the high-dose female mice.

Increased liver tumour incidences were also observed in male and female B6C3 mice administered 30 or 60 μg 2,3,7,8-TCDD/kg bw intraperitoneally in corn oil once weekly for 5 weeks beginning at 5 days of age, or 2.5, or 5 μg/kg by corn oil gavage from 6 weeks of age for 52 weeks with observation until week 110 (Della Porta *et al.*, 1987).

In male Syrian Golden hamsters, a total dose of 600 μg 2,3,7,8-TCDD/kg bw administered over a 4-week period by either the intraperitoneal or the subcutaneous route resulted in a 20 percent incidence of facial squamous cell carcinomas after 12 to 13 months (Rao *et al.*, 1988).

A mixture of two hexachlorodibenzodioxins (1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD) was administered twice per week to Osborne Mendel rats and B6C3F1 mice. Rats were administered 0, 1.25, 2.5, or 5 μg HxCDD/kg bw, as were male mice, while female mice were administered 0, 2.5, 5, or 10 μg HxCDD/kg. An increased incidence of hepatic neoplasms was observed in both sexes of both species with the female rat being the most sensitive (NTP, 1980; Kociba and Schwetz, 1982).

Mutagenicity tests with 2,3,7,8-TCDD have in general been negative (Wassom *et al.*, 1977; Shu *et al.*, 1987) and 2,3,7,8-TCDD does not bind covalently to DNA (Poland and Glover, 1979; Turtletaub *et al.*, 1990; Randerath *et al.*, 1988, 1990). Actually, 2,3,7,8-TCDD administration decreased the number of endogenous adducts (I-compounds) in female, but not male, Sprague-Dawley rats. Furthermore, 1,2,3,7,8-PnCDD decreased, while 1,2,4,7,8-PnCDD failed to alter the endogenous I-compound levels in the liver (Randerath *et al.*, 1990).

Studies in the mouse skin support a lack of initiating activity, but 2,3,7,8-TCDD has shown strong tumour promoting activity for epidermal carcinogenesis in hairless mice (Poland *et al.*, 1982; Shu *et al.*, 1987; Hebert *et al.*, 1990a). Other PnCDD and PnCDF congeners, which bind to the Ah receptor also promoted skin tumours, while 2,7-DCDD did not act as a tumour promoter (Poland *et al.*, 1982; Poland and Glover, 1986; Hebert *et al.*, 1990b).

Many studies have also shown that 2,3,7,8-TCDD is a potent tumour-promoting agent in the female rat liver after partial hepatectomy and initiation with diethylnitrosamine (DEN) (Pitot *et al.*, 1980; Dragan *et al.*, 1992; Graham *et al.*, 1988; Flodstrom and Ahlborg, 1989, Hendrich *et al.*, 1987; Maronpot *et al.*, 1993; Pitot *et al*., 1987; Clark *et al.*, 1991). Several of these studies also indicated the independence of enzyme induction from tumour promotion. Stinchcombe *et al.* (1995) found that chronic administration of 2,3,7,8-TCDD to rats resulted in an increase in focal hepatic labelling index and a marked decrease in focal apoptosis.

Several compounds that are structurally related to 2,3,7,8-TCDD have been examined with respect to their tumour-promoting activity in the female rat liver. 2,3,7,8-TCDD and 1,2,3,7,8-PnCDD were equipotent in the promotion of liver foci, while 2,3,4,7,8-PnCDF was less than one-tenth as potent (Wærn *et al.*, 1991; Flodstrom and Ahlborg, 1991). Schrenk *et al.* (1994) and Buchmann *et al.* (1994) found 1,2,3,4,6,7,8-HpCDD to be a liver tumour promoter in female rats, while Nishizumi and Masuda (1986) have shown that 2,3,4,7,8-PnCDF and 1,2,3,4,7,8-HxCDF were effective in five-week-old male Wistar rats. Hemming *et al.* (1995) showed an additive effect of 2,3,7,8-TCDD and 3,3',4,4',5- PCB (PCB 126) on liver tumour promotion in female Sprague Dawley rats.

Lucier *et al.* (1991) found the induction of altered hepatic foci attenuated in ovariectomised female Sprague-Dawley rats. It was therefore suggested that oestrogen may contribute to the liver carcinogenicity observed in female rats with 2,3,7,8-TCDD (Huff *et al.*, 1994). Alternative suggestions include the induction of hepatotoxicity with the dose of 2,3,7,8-TCDD that induces both hepatic tumour promotion and hepatocarcinogenesis in female rats (Rozman *et al.*, 1993; Jensen *et al.*, 1983; Goodman and Sauer, 1992).

2. Human data

2.1. Non-cancer effects (based on Sweeney and Mocarelli, 1998)

Among the many populations studied to examine the relationship between non-cancer health effects and exposure to 2,3,7,8-TCDD contaminated chemicals, biologic measures of exposure were only available for chemical workers in the US and Germany; Air Force Ranch Hands, Army Vietnam Veterans; and residents of Missouri, U.S.A. and Seveso, Italy.

Chloracne is the most widely recognised dermal effect of exposure to 2,3,7,8-TCDD-contaminated substances. Chloracne has been observed in some workers after all reported accidents at trichlorophenol (TCP) production facilities (Zober *et al.*, 1990) and among individuals involved in production of 2,3,7,8-TCDD-contaminated products (Bond *et al.*, 1989). At least 193 Seveso residents, mostly children, experienced chloracne; however, the condition disappeared after discontinuation of exposure despite high serum 2,3,7,8-TCDD levels ranging from 820 to 56,000 pg/g lipid measured within 1 year of the accident (Mocarelli *et al.*, 1986; Assennato *et al.*, 1989; Mocarelli *et al.*, 1991). Other individuals from Zone A, but without chloracne, had serum 2,3,7,8-TCDD levels that ranged from 1,770 to 10,400 pg/g lipid. Chloracne has also been induced in cows, horses, on the ears of rabbits, and on the skin of hairless mice.

Children (particularly boys) in Seveso experienced an increase in serum gamma glutamyl transferase (GGT) levels occurring shortly after the accident and then a gradual decline to near normal levels within 5 years. Elevations in serum ALT appeared to be a transient effect of acute exposure to 2,3,7,8-TCDD in Seveso (Mocarelli *et al.*, 1986). A similar pattern of elevations in GGT have been noted among TCP production workers with high serum 2,3,7,8-TCDD levels (>100 pg/g fat) and high alcohol consumption (Calvert *et al.*, 1992).

In 1978, D-glucaric acid excretion was significantly elevated in adults residing in Seveso at the time of the accident. In 1976, the levels in children from Zone A with chloracne were significantly greater than in children without chloracne. Additional studies, conducted until 1981, found significant yearly decreases in urinary D-glucaric acid excretion. By 1981, levels were within the normal range (Ideo *et al.*, 1985).

Evidence of alterations in porphyrin metabolism among populations exposed to 2,3,7,8-TCDD is inconsistent (Calvert *et al.*, 1994).

In general, cholesterol levels among exposed TCP production workers or residents of Seveso were not increased (Ott *et al.*, 1994; Calvert *et al.*, 1996; Mocarelli *et al.*, 1986; Assennato *et al.*, 1989). A positive relationship between serum 2,3,7,8-TCDD levels above 33.3 pg/g fat and total cholesterol has been reported in Air Force Ranch Hands (Roegner *et al.*, 1991). In the 1992 analysis, the difference was not great enough to achieve statistical significance (Grubbs *et al.*, 1995).

Among workers in the NIOSH study there appeared to be a small rise in triglyceride levels with increasing serum 2,3,7,8-TCDD (Calvert *et al.*, 1996). However, the influence of gender, body mass index, use of beta-blocker medication, and smoking had far greater effects on lipid concentration than the 2,3,7,8-TCDD level. Triglyceride levels in the BASF accident cohort were similar to those in the referent cohort and not related to the 2,3,7,8-TCDD level (Ott *et al.*, 1994). Triglyceride concentrations were elevated among Ranch Hands having the highest

serum 2,3,7,8-TCDD levels (Grubbs *et al.*, 1995). Triglyceride levels were not elevated in Seveso residents (Mocarelli *et al.*, 1986; Assennato *et al.*, 1989).

Most studies of exposed humans have found parameters of thyroid function, e.g. THS, T4 and thyroxine-binding globulin (TBG), within the normal range (Ott *et al.*, 1994; Calvert *et al.*, submitted) although in some studies their levels showed relations to 2,3,7,8-TCDD levels in regression analyses (Ott *et al.*, 1994; Calvert *et al.*, 1999; Roegner *et al.*, 1991; Grubbs *et al.*, 1995).

In linear regression analyses, serum 2,3,7,8-TCDD was positively and significantly related to serum concentration of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and inversely related to total testosterone among TCP production workers (Egeland *et al.*, 1994). No association with serum 2,3,7,8-TCDD was observed for testosterone, FSH, LH, testicular abnormalities or volume, or sperm count or abnormalities among Ranch Hand veterans (Henriksen *et al.*, 1996).

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 2,3,7,8-TCDD contaminated area in Seveso (Mocarelli *et al.*, 1996). An explanation of this phenomenon has not been offered but a possible role of hormonal disruption cannot be ruled out.

In a German cohort involved in a reactor accident in TCP production (BASF), the mean fasting glucose levels were marginally elevated compared to the referent population and associated with concentrations of 2,3,7,8-TCDD at the time of the study but not the concentration estimated at the time of last exposure (Ott *et al.*, 1994). Particpants in the Ranch Hand study with 2,3,7,8-TCDD concentrations ≥94 pg/g lipid were at increased risk for diabetes (Henriksen *et al.*, 1997). Among US TCP production workers, the overall prevalence of diabetes mellitus was not significantly different from controls and there was no significant trend between prevalence of diabetes and increasing serum 2,3,7,8-TCDD concentration (Calvert *et al.*, 1999). However, diabetes was found in 6 of 10 workers with serum 2,3,7,8-TCDD concentrations in excess of 1500 pg/g lipid.

Immunocompetence was tested twice in 44 children who were residents of the region of Seveso with the highest 2,3,7,8-TCDD contamination. It was reported that the various measures were within the normal range (Reggiani, 1978). In a study of Missouri residents, Webb *et al.* (1989) found no clinical evidence of immunosuppression in 40 individuals whose adipose 2,3,7,8-TCDD levels ranged from under 20 pg/g to over 430 pg/g. Tests included serum immunoglobulins, T-cell surface markers OKT3, OKT4, OKT8, OKT11, Leu11c, CD4/CD8 ratio, (CD4 + CD8)/CD3, and B1 and B2 cells. In logistic regression, significant ($p<0.05$) relationships were noted for IgG, %CD3, %T11, %CD8, and %CD4 + LEU8 POS, controlling for age and sex.

Among participants in the BASF accident study cohort, with the exception of natural killer cells and helper-inducer cells, the proportions of some lymphocyte populations (B cells, T cells, T helper cells, T suppressor cells) were lower among workers, but the distribution of cells in referents and workers was equivalent (Ott *et al.*, 1994). Levels for IgA, IgG, IgM, and complement C4 and C3 were slightly higher in these workers. In 18 British workers who were evaluated 17 years after accidental industrial exposure to chemicals contaminated with 2,3,7,8-TCDD there were no significant differences in the levels of immunoglobulins, T and B lymphocytes, responsiveness to phytohemagglutinin A, and in the number of CD4 and CD8

counts. Three measures were found to be higher in these workers, namely antinuclear antibodies, immune complexes and natural killer cells (Jennings *et al.*, 1988).

No significant differences were noted among U.S. Army ground troops and the comparison population in lymphocyte subset populations, T-cell populations, or serum immunoglobulins (Centers for Disease Control Vietnam Experience Study, 1988a). Comprehensive immunologic profiles were developed for each participant of the U.S. Air Force Ranch Hand Study (Roegner *et al.*, 1991) and no 2,3,7,8-TCDD related changes were seen.

Numerous case reports cite symptoms referable to the nervous system occurring after acute as well as chronic exposure to 2,3,7,8-TCDD-contaminated materials. Symptoms include headache, insomnia, nervousness or irritability, depression and anxiety, loss of libido, and encephalopathy.

A number of studies have examined possible neurobehavioural effects among various exposed populations, however, direct comparison of the results from the various studies is difficult. Serum 2,3,7,8-TCDD levels were not associated with depressed mood in the NIOSH cross-sectional medical study (Alderfer *et al.*, 1992) and in the U.S. Air Force study (Roegner *et al.*, 1991) of personnel who applied Agent Orange during the Vietnam war. In addition, in the Ranch Hand Study there was no relationship with serum 2,3,7,8-TCDD levels and psychological illness or sleep disorders.

No relationship between 2,3,7,8-TCDD concentration and differences in neurologic status or nerve function was found among TCP workers (Sweeney *et al.*, 1993). Ranch Hand veterans with serum 2,3,7,8-TCDD concentrations above 33.3 pg/g fat tended to have a higher proportion of individuals with abnormal coordination than the comparison group (Roegner *et al.*, 1991). Overall neurologic status of US Army Vietnam veterans did not differ from that of non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988b). In residents of Seveso who developed chloracne, no increases were found in the prevalence of abnormal electrophysiologic measures or in conduction velocities (Assennato *et al.*, 1989).

In a number of studies, mortality from all diseases of the circulatory system among TCP production workers was similar to mortality in the general population (Bueno de Mesquita *et al.*, 1993; Collins *et al.*, 1993, Coggon *et al.*, 1991; Bond *et al.*, 1987; Zober *et al.*, 1990). In a study of German workers, who manufactured 2,4,5-TCP and 2,4,5-T in addition to chemicals contaminated with higher chlorinated PCDDs and PCDFs, mortality for all circulatory diseases was positively related to 2,3,7,8-TCDD levels and significantly related to estimated total TEQ concentrations above 39 ng/kg lipid (Flesch-Janys *et al.*, 1995). Mortality from ischaemic heart disease (IHD) was significantly increased among the German workers only in the highest 2,3,7,8-TCDD quintile (Flesch-Janys *et al.*, 1995). Among 1,261 Ranch Hand personnel, mortality from circulatory disease was nonsignificantly elevated (Michalek *et al.*, 1990). These results were repeated in an updated mortality analysis (Wolfe *et al.*, 1994). Similar nonsignificant increases in mortality were observed from circulatory diseases in Australian Vietnam veterans (Fett *et al.*, 1987). In contrast, a relative risk of 0.49 for all circulatory diseases was found among US Vietnam Army veterans (Centers for Disease Control Vietnam Experience Study, 1988c). The relative mortality risks for circulatory system diseases reported in the majority of the studies are close to 100, suggesting that the "healthy worker effect" is not seen in these studies. These results suggests that more detailed analyses should be conducted for cardiovascular outcomes.

Bertazzi and colleagues examined the mortality among Seveso residents 10 years after the contamination (Bertazzi *et al.*, 1989, 1992). Circulatory disease mortality of residents from Zone A (the most highly contaminated region) was elevated in both males and females. The small number of subjects and the crude measure of 2,3,7,8-TCDD exposure limited the study. The authors suggested that the "high stress and pollution" imposed on the residents of Zone A may have been a contributing factor.

There is conflicting evidence from controlled epidemiologic studies regarding an association between chronic respiratory system effects and human exposure to substances contaminated with 2,3,7,8-TCDD (Suskind and Hertzberg, 1984; Calvert *et al.*, 1991; Roegner *et al.*, 1991; Grubbs *et al.*, 1995).

There is little evidence to suggest that exposure to 2,3,7,8-TCDD is related to renal or bladder dysfunction (Roegner *et al.*, 1991; Suskind and Hertzberg, 1984).

Among Yusho and Yucheng adults, chronic exposure related effects included chloracne, conjunctivitis, and sebaceous cysts and inflammation, decreased nerve conduction, fatigue and malaise, hyperpigmentation and hyperkeratosis, and increased mortality from non-malignant liver disease (Kuratsune *et al*., 1996).

2.2. Effects in children after prenatal exposure (based on Feeley and Brouwer, 1998)

Two US birth cohorts with measured background exposure to PCBs have been followed since 1980, and 2 Dutch birth cohorts with measured background levels of PCBs, PCDDs and PCDFs have been followed since 1990. In Asia, data are available on Japanese children exposed transplacentally to contaminated rice oil, and detailed follow-up is available on transplacentally exposed children in Taiwan.

Severe developmental effects were observed in non-breastfed infants and children born to Yu-cheng mothers with estimated body burdens of 2-3 μg I-TEQ/kg body weight based on PCDFs and dioxin-like PCBs (Needham, 1993). The developmental effects included low birth weight, hyperpigmentation, increased incidence of skin and respiratory infections, neurological dysfunction, natal teeth and alterations in sexual development (Rogan *et al.*, 1988; Chen *et al.*, 1992; Chen and Hsu, 1994). The neurobehavioural changes consisted of hypotony, hyperactivity, lower mean intelligence quotients and altered latencies and amplitudes of auditory event-related potentials. Analysis of placental samples collected from mothers affected by the Yu-cheng incident 5 years following exposure revealed 610-9010 ng I-TEQ/kg lipid. Average concentrations seen in North American placentas were 8.4-17.6 ng I-TEQ/kg lipid (Schecter *et al.*, 1996b). Lowest intake of I-TEQs estimated to result in minimal Yusho symptoms was 28 ng/kg bw/day for 135 days.

The Michigan Maternal/Infant Cohort study reported that women who consumed fish from Lake Michigan prior to and during pregnancy delivered infants who, on average, had lower birth weight (160-190 g) and had smaller head circumferences (0.6-0.7 cm) than infants born to women who did not eat Great Lakes fish (Fein *et al.*, 1984). At 4 years of age the weight difference was still apparent (Schantz, 1996). High intake of fish from the Baltic Sea has been described as a possible risk factor for having infants with low birth weight (Rylander *et al.*, 1995, 1996) and in the Dutch PCB/Dioxin cohort the I-TEQ level of dioxins and PCBs in breast milk was negatively associated with infant birth weight (Huisman *et al.*, 1995a, 1995b).

However, in another study involving Lake Michigan sport fish consumers, mean infant birth weight was positively associated with reported maternal fish consumption and PCB exposure (Dar *et al.*, 1992).

Some of the children from the Michigan cohort exhibited motoric immaturity, hypoactive reflexes and reduced scores on a variety of tests designed to assess memory, cognitive function and IQ. Associations between cord serum PCB levels and the scores in the behavioural tests were only seen in children of the top 11% of the fish-consumers with breast milk PCB concentrations in excess of 1.25 mg/kg lipid. Testing when the children were 11 years of age indicated that prenatal exposure to PCBs (breast milk PCB concentrations equal to and greater than 1.25 mg/kg lipid) is associated with a 6.2-point deficit in infant full-scale IQ (Jacobson *et al.*, 1990a, 1990b; Jacobson and Jacobson, 1996, 1997; Schantz, 1996).

In the North Carolina Breast Milk and Formula Project the group of infants born to mothers with the highest PCB concentration in breast milk (greater than 3.5 μg/g lipid) were described as being hypotonic and hyporeflexive when tested at birth. A significant relation was also found between prenatal PCB exposure and poorer psychomotor performance at 6, 12 and 24 months of age (Gladen *et al.*, 1988). The effect was only seen in the most highly exposed infants. These initial delays in psychomotor development were transient by the age of 2, and between the ages of 5.5 and 10.5 years no association was seen (Gladen and Rogan, 1991).

A number of methodological questions have been raised concerning, in particular, the Michigan cohort (exposure assessment difficulties, control group selection, confounding variables) and other explanations include exposure to different contaminants and socio-demographic variables (Paneth, 1991). In addition, the IQ score reductions seen in the more heavily exposed Yu-cheng infants were of a similar magnitude. This weakens any conclusions (Middaugh and Egeland, 1997).

In the Dutch PCB/Dioxin breast milk study the mean I-TEQ concentrations in human milk were 30.2 pg/g lipid (range 11.1-76.4 pg/g). Inclusion of the TEQ contribution from PCBs increased the "total" TEQ concentration approximately 100% (65.7 pg/g lipid mean) (Koopman-Esseboom *et al.*, 1994a, 1994c; Huisman *et al.*, 1995a, 1995b). A positive dose-dependent association was observed between infant plasma TSH levels in the second week and third month after birth and the human milk TEQs. Infants exposed to higher than average human milk TEQ levels had significantly lower plasma FT4 and TT4 levels than infants exposed to lower than average human milk TEQs (Koopman-Esseboom *et al.*, 1994b).

Infant neurodevelopmental testing indicated that there was no relationship between infant visual recognition memory at 3 months of age and human milk TEQs, or maternal or cord plasma PCB levels at 3 and 7 months of age. At 7 months of age the breast-fed infants had significantly higher scores compared to the formula-fed infants when they received breast feeding for a longer period, regardless of cumulative exposure to I-TEQs in breast milk (Koopman-Esseboom, 1996). These data are in contrast to results in the Michigan cohort (Jacobson *et al.*, 1985). In addition, the psychomotor scale (PDI) of the Bayley test at 3 months of age was negatively correlated with cord plasma PCB levels (Koopman-Esseboom *et al.*, 1996). At 7 months of age the mental development scale (MDI) and PDI were positively associated with the duration of breast-feeding. However, when breast-fed infants received higher cumulative TEQ levels *via* human milk the positive effect of breast feeding on the PDI diminished. No associations were observed between PDI or MDI scores at 18 months of age and human milk TEQs of dioxin-like PCBs, PCDDs and PCDFs. Significant negative associations were found between the neurological optimality score (NOS) and the

human milk TEQs. Logistic regression analysis demonstrated a significantly higher percentage of hypotonia with higher planar PCB TEQs in human milk and a tendency to hypotonia with higher PCDD/PCDF and PCB TEQs in human milk (Huisman *et al.*, 1995b).

In another Dutch study, the children were divided into two groups based on milk I-TEQs; low exposure group mean 18.6 ng/kg lipid, range 8.7-28.0 ng/kg and high exposure group mean 37.5 ng/kg lipid, range 29.2-62.7 ng/kg (Pluim *et al.*, 1993). In cord blood samples, none of the thyroid hormone measurements differed between the two groups while TT4 levels were significantly elevated in the high exposure group at 1 week and 11 weeks of age. In addition, TSH levels were elevated in the high exposure group at 11 weeks of age. When children were tested at 2 years of age with the Bayley Scales of Infant Development (PDI and MDI), mean PDI scores tended to be higher in the high cumulative I-TEQ intake group, but not significantly (Ilsen *et al.*, 1996). When a neurological examination of the children was performed at the age of 2 years and 7 months, there were indications of enhanced neuromotor maturation and higher reflexes in the children from the high exposure group (29.2-62.7 ng I-TEQ/kg milk lipid, mean 37.4 ng/kg).

2.3. Carcinogenicity (based on Kogevinas, 1998)

In most of the human studies examining the carcinogenicity of dioxins, people were exposed to mixtures of PCDDs, including 2,3,7,8-TCDD, as contaminants of phenoxy herbicides and chlorophenols. These epidemiological studies have involved subjects with the highest recorded exposures to 2,3,7,8-TCDD.

In a large study involving 12 industrial plants in the USA the blood lipid 2,3,7,8-TCDD levels estimated to the last time of exposure were 2000 ng/kg lipid (mean) (up to 32,000 ng/kg lipid). Mortality from all cancers was slightly but significantly elevated. Soft-tissue sarcoma mortality was elevated based on four deaths (Fingerhut *et al.*, 1991b). Among two groups of workers in The Netherlands, the blood lipid 2,3,7,8-TCDD concentration at time of exposure was estimated to be 1434 ng/kg (geometric mean) (range 301-3683 ng/kg) in those involved in the clean up of a reactor explosion (Bueno de Mesquita *et al.*, 1993; Hooiveld *et al.*, 1998). In these groups all-cancer mortality was significantly increased. Excesses at specific sites were seen for urinary bladder, kidney and non-Hodgkin lymphoma. A group of German workers with severe chloracne after an accident at a trichlorophenol production unit showed 1008 ng/kg lipid (geometric mean) (Ott *et al.*, 1993). Increased all-cancer mortality with increasing dose was apparent. Digestive system and respiratory tract cancers also tended to increase with increasing dose (Zober *et al.*, 1990; Ott and Zober, 1996). Several other German cohorts have been followed, and measurements up to 2252 ng/kg lipid have been made in a cohort from Boehringer. Mortality increases were seen for all cancers combined, cancer of the buccal cavity and pharynx, lung cancer, lymphatic and haematopoietic neoplasms and non-Hodgkin lymphoma (Becher *et al.*, 1996; Kogevinas *et al.*, 1997). Several studies on the cohort of workers from Boehringer found that mortality due to all cancers combined was increased and mortality from breast cancer in females was higher than expected (Manz *et al.*, 1991; Nagel *et al.*, 1994).

Exposures in Seveso (median in zone A back-calculated to exposure date in 1976, 389 ng/kg lipid; median in Zone B, 78 ng/kg lipid) were, on average, lower than those of the industrial cohorts. The upper range of the high exposed individuals were similar to that of the occupational cohorts (upper 75th percentile in Zone A, about 2000 ng/kg) (Landi *et al.*, 1987). At 15 years of follow-up, all-cause and all-cancer mortality did not differ significantly from

those expected in any of the contaminated zones. Mortality from gastrointestinal cancer was increased. Women had an increased mortality from stomach cancer, and men had increased mortality from rectal cancer. Mortality from various haematological neoplasms was also increased. The highest risks were seen in Zone B for leukaemia in men, multiple myeloma in women, and Hodgkin's disease in both genders. There was no increase in mortality from major cancer sites such as respiratory tract among males, and breast in females (Bertazzi *et al.*, 1997).

Two studies have evaluated cancer risk among subjects exposed to contaminated rice oil in Japan (Yusho) and Taiwan (Yucheng). The contaminated rice oils contained a complex mixture of PCDFs, dioxin- and non-dioxin-like PCBs, polychlorinated quaterphenyls and polychlorinated terphenyls. The Japanese oil contained of the order of 1000 ppm PCBs and 5 ppm PCDFs and was consumed over about a month. The daily intake was estimated at 0.33 mg PCB/kg bw/day and 0.002 mg PCDF/kg bw/day. The Taiwanese oil contained about 100 ppm PCBs and 0.4 ppm PCDFs and was consumed over about 10 months. The daily intake was estimated at 0.06 mg PCB/kg bw/day and 0.0002 mg PCDF/kg bw/day. There was an excess liver cancer risk in Japan at 22 years of follow-up, and no excess risk in Taiwan at 12 years (Kogevinas, 1998).

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ANNEX III

BACKGROUND, CONCEPTS AND STUDIES CONSIDERED IN THE RISK ASSESSMENT OF DIOXINS AND DIOXIN-LIKE PCBS IN FOOD

Table of Contents

1. Introduction

This annex contains a discussion on the background, concepts and studies considered in the risk assessment of dioxins and dioxin-like PCBs in food. In addition previous risk assessments of dioxins and the concept of using toxic equivalency factors is mentioned. The discussion mainly relates to the most recent risk assessment of dioxins and dioxin-like PCB performed by a WHO Consultation in 1998 (WHO, 1998; WHO, 2000).

2. Background

2.1. Previous assessments of PCDDs, PCDFs and dioxin-like PCBs

Two different approaches have been used world-wide in the risk assessments of PCDDs, PCDFs and dioxin-like PCBs. WHO and most countries outside the USA have derived Tolerable Daily (or weekly) intakes (TDI) for TCDD in the range of 1-10 pg/kg bw/day, assuming the existence of a threshold dose for the carcinogenic effect of TCDD (see Table 1). In contrast, US EPA and FDA have used probabilistic estimates of cancer potency, treating cancer as a non-threshold effect, in the derivation of a Risk Specific Dose (RsD) as low as 0.006 pg/kg bw/day (US EPA, 1989, 1994). The most recent risk assessment of dioxins and dioxin-like PCBs has been performed by WHO in 1998 (WHO, 1998).

The complex nature of mixtures of PCDDs, PCDFs, and PCBs complicates the risk evaluation for humans. To facilitate risk assessment and regulatory control of exposure to these mixtures the concept of toxic equivalency factors (TEFs) has been developed. The TEF values express the toxic potencies of the other congeners relative to that of 2,3,7,8-TCDD (TCDD). TEF values for individual congeners in combination with their chemical concentration can be used to calculate the total TCDD toxic equivalents concentration (TEQs) contributed by all dioxin-like congeners in the mixture using the following equation:

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

Several different TEF schemes have been developed. Until now the most widely used schemes have been the International TEFs (I-TEFs) (NATO/CCMS, 1988) for PCDDs and PCDFs, and the WHO-ECEH (European Centre for Environment and Health of the World Health Organization) scheme for PCBs (Ahlborg *et al.*, 1994). In June 1997, WHO-ECEH and the International Programme on Chemical Safety (IPCS) arranged an international meeting in Stockholm, which resulted in the consensus of TEFs for PCDDs, PCDFs and dioxin-like PCBs for both human (WHO-TEFs, Table 2), fish and wildlife risk assessment (van den Berg *et al.*, 1998). TEFs for dioxin-like compounds apply only to AhR-mediated responses. The criteria used by WHO for including a compound in the TEF scheme for dioxin-like compounds were that the compound must:

- Show a structural relationship to the PCDDs and PCDFs.
- Bind to the Ah receptor.
- Elicit Ah receptor-mediated biochemical and toxic responses.
- Be persistent and accumulate in the food chain.

To determine TEFs for mammals WHO followed a tiered approach in which results of animal toxicity studies, especially those involving (sub)chronic exposure, were given significantly more weight than results of *in vitro* or biochemical studies (van den Berg *et al.*, 1998).

The use of this concept assumes dose additivity. While additivity predominates in the majority of experimental studies, non-additive interactions of PCDD, PCDF and PCB mixtures have been reported at greater than environmental levels of exposure. In particular, antagonistic effects have been reported. These non-additive effects are considered to be due to multiple mechanisms of action of individual congeners and/or to pharmacokinetic interactions. For the mono-*ortho* PCBs especially, certain endpoints such as carcinogenicity, porphyrin accumulation, alterations in circulating thyroid hormone concentrations and neurotoxicity

Table 1. Examples of risk assessments of TCDD and TEF schemes recommended

Country or organisation	**TDI for TCDD pg/kg bw/day**	**Basis for evaluation**	**TEF values recommended**
The Netherlands, 1982 (Van der Heijden *et al*., 1982)	4	Marginal LOAEL of 1 ng/kg bw/d in chronic toxicity, carcinogenicity and multigeneration reproduction studies in rats. Safety factor of 250	-
Germany, 1984 (UBA, 1985)	1-10	NOAEL of 1 ng/kg bw/d in chronic toxicity, carcinogenicity and multigeneration reproduction studies in rats. Safety factor of 100-1000	German TEFs (UBA, 1985). Germany, in 1990, adopted I-TEFs (Dioxin Symp., 1993)
Nordic countries, 1988 (Ahlborg *et al.*, 1988)	5 (expressed as 35 pg/kg bw/week due to the cumulative properties of these compounds)	Marginal LOAEL of 1 ng/kg bw/d in chronic toxicity, carcinogenicity and multigeneration reproduction studies in rats. Safety factor of 200	N-TEFs (Ahlborg *et al.*, 1988)
NATO/CCMS (1988)	-	Available *in vitro* and *in vivo* studies	I-TEFs
UK, 1989 (DH, 1989)	1 (guideline value, not TDI)	NOELs from available animal studies on immunotoxicity, reproductive toxicity and carcinogenicity	I-TEFs
WHO/EURO, 1990 (Ahlborg *et al.*, 1992)	10	The steady state liver TCDD concentration of 540 ppt in rats at NOAEL of 1 ng/kg bw/day (Kociba *et al.*, 1978) corresponds to a human daily intake of 110 pg TCDD/kg bw/day. Safety factor of 10.	I-TEFs
The Netherlands, 1991 (Liem and Theelen, 1997)	10	Adoption of WHO 1991 evaluation	I-TEFs
UK, 1991 (MAFF, 1992)	10	WHO 1991 evaluation endorsed	I-TEFs
WHO, 1993 (Ahlborg *et al.*, 1994)	-	Available *in vitro* and *in vivo* studies	PCB-TEFs for dioxin-like PCBs
Health Council of The Netherlands (1996)	1	LOAEL of 100 pg/kg bw/d (maternal) in monkeys for changes in cognitive recognition (offspring), endometriosis and white blood cells. Safety factor of 100.	I-TEFs and PCB-TEFs
WHO, 1997 (van den Berg *et al.*, 1998)	-	Available *in vitro* and *in vivo* studies	WHO-TEFs for dioxins and dioxin-like PCBs
UK, 1998 (Committee on Toxicity, 2000)	-	WHO 1997 evaluation endorsed	Adoption of WHO-TEFs
WHO, 1998 (WHO, 2000)	1-4 (as WHO-TEQ)	Various sensitive non-cancer endpoints in experimental animals. Evaluation based on estimates of body burdens converted to equivalent human intakes	WHO TEFs

could arise by both Ah receptor-mediated and non-Ah receptor-mediated mechanisms. This increases the uncertainty in the use of TEFs for mono-*ortho* PCBs. In addition, non-Ah receptor-mediated mechanisms of action may be shared by certain di-, tri-, and tetra-chloro *ortho*-substituted PCBs normally also present in the environment and food (van den Berg *et al.*, 1998).

WHO stressed that the TEF indicates an *order of magnitude* estimate of the toxicity of a compound relative to TCDD using careful scientific judgement after considering all available data.

Table 2. WHO TEFs for human risk assessment (van den Berg *et al.*, 1998).

Congener	TEF value	Congener	TEF value
Dibenzo-p-dioxins		***Non-ortho PCBs***	
2,3,7,8-TCDD	1	PCB 77	0.0001
1,2,3,7,8-PnCDD	1	PCB 81	0.0001
1,2,3,4,7,8-HxCDD	0.1	PCB 126	0.1
1,2,3,6,7,8-HxCDD	0.1	PCB 169	0.01
1,2,3,7,8,9-HxCDD	0.1		
1,2,3,4,6,7,8-HpCDD	0.01	***Mono-ortho PCBs***	
OCDD	0.0001	PCB 105	0.0001
		PCB 114	0.0005
Dibenzofurans		PCB 118	0.0001
2,3,7,8-TCDF	0.1	PCB 123	0.0001
1,2,3,7,8-PnCDF	0.05	PCB 156	0.0005
2,3,4,7,8-PnCDF	0.5	PCB 157	0.0005
1,2,3,4,7,8-HxCDF	0.1	PCB 167	0.00001
1,2,3,6,7,8-HxCDF	0.1	PCB 189	0.0001
1,2,3,7,8,9-HxCDF	0.1		
2,3,4,6,7,8-HxCDF	0.1		
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0001		

2.2. Key points in the re-evaluation performed by the WHO Consultation in 1998

The Committee wishes to acknowledge the WHO evaluation for providing a scientifically sound framework for the risk assessment of mixtures of compounds such as dioxins that accumulates in the body of experimental animals and humans. The key points in the WHO evaluation were:

- The WHO group used (estimated) body burdens as dose metrics rather than the daily doses in order to compare across species.

- WHO deviated from some earlier evaluations of dioxins in not using carcinogenicity as the critical endpoint for the final TDI derivation.

- Although studies in humans were also carefully evaluated, the WHO group based its evaluation on studies in experimental animals.

- WHO identified developmental, reproductive and hormonal effects in rats and monkeys following exposure to TCDD as the most sensitive adverse effects reported, i.e. the adverse effects occurring at the lowest body burdens.

- WHO advocated the use of the most up-to-date TEF scheme for dioxins and dioxin-like PCBs, i.e. WHO TEFs (van den Berg *et al.*, 1998) to calculate the total TEQ in mixtures of these compounds.

- Based on the evaluation of TCDD, WHO expressed the TDI as 1-4 pg WHO-TEQ/kg bw.

In the following sections, the Committee discusses the principles used in the WHO assessment and has performed a re-evaluation of the critical studies that were used for the establishment of the tolerable intake. Relevant information from studies that have become available since the WHO Consultation in 1998 has been included.

3. Use of body burden as dose metric

Many of the effects following dioxin exposure are the same irrespective of whether the intake is acute or chronic. For effects such as enzyme induction and immunotoxicity (van Birgelen *et al.*, 1997), developmental effects (Birnbaum, 1991) and a number of other toxic endpoints (Viluksela *et al.*, 1997a, 1997b) the responses are directly associated with tissue concentrations and not with the daily dose. The key determinants in the kinetics and the half-lives of these compounds are amount of fat stores in the body, binding to CYP1A2 in the liver, and rate of metabolism and excretion. Due to differences in these determinants (Li and Rozman, 1995; van Birgelen *et al.*, 1997), in particular in rate of metabolism and excretion, rodent species require appreciably higher doses (100-200-fold) to reach the same equivalent body burdens as seen in humans at background exposures. From a pharmacokinetic point of view, body burden estimations are therefore considered a more appropriate dose metric for interspecies comparison than the daily dose.

Limited information is available about differences in sensitivity between humans and laboratory animal species. PCDDs, PCDFs, and the dioxin-like PCBs exert a number of biochemical and toxicological effects mediated through the Ah receptor. Studies of Ah receptor binding affinity and responses directly dependent on Ah receptor activation suggest that humans may be less susceptible to TCDD than responsive rodent strains, whereas studies of other biochemical or cellular effects suggest comparative susceptibility (DeVito *et al.*, 1995). *In vitro* studies using liver slices suggest that rats and humans show similar sensitivity to TCDD as regards CYP1A1 and 1A2 induction, at least within an order of magnitude, when based on the liver concentration (Drahushuk *et al.*, 1997). The body burden associated with many of the biochemical and toxicological responses to TCDD exposure is similar across species. Thus, for a number of effects such as chloracne and induction of CYP1A1, humans respond at similar body burdens to animals (DeVito *et al.*, 1995). Comparison of the relative susceptibility of animals and humans to cancer induced by TCDD indicates that humans are, at most, a factor of 10 less sensitive than rodents based on average lifetime serum lipid TCDD concentrations or lifetime area-under-the-curve (Aylward *et al.*, 1996). This also indicates that tissue derived dosimeters should be the base for risk assessment of dioxin-like compounds (Santostefano *et al.*, 1998).

The most appropriate dose metric would ideally be the concentration at the target tissue. However, it is seldom known. WHO therefore used (estimated) body burdens of TCDD in the experimental animals of the critical studies as the key dose metrics. In order to transform these animal body burdens into estimated daily human intakes that on a chronic basis would lead to similar body burdens in humans (at steady state) WHO used simple classical pharmacokinetic calculations. The half-life of elimination of dioxins is a first-order process, which is independent of the body burden or dose. Under steady state conditions, the relation between the total body burden and intake is as follows:

Body Burden (ng/kg bw) = f * Intake (ng/kg bw/day) * half-life/ln(2)

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 7.5 years (2740 days).

This model assumes that dioxins are distributed in only one compartment (the whole body). Although the majority of the dioxin mass is distributed to the lipid stores of the body in animals and humans it has been found that animals and humans sequester these compounds in the liver at higher doses. This clearly indicates that it is necessary to include factors other than lipid solubility in PBPK models for an accurate prediction of tissue distribution in both rodents and humans, especially at higher exposures. However, the Committee finds that the approach taken by WHO by comparing animal and human body burdens and using simple classical pharmacokinetics for the calculation of estimated equivalent human intakes is appropriate, in particular in relation to exposures in the low dose range as is the case for the general human population. In support, DeVito *et al.* (1995) found that such a simple model provided reasonable accurate estimates of body burdens in rats that received between 1 and 100 ng TCDD/kg bw/day in semichronic studies when compared with estimates based on measurements of actual tissue concentrations.

4. Effects considered, but not pivotal for the derivation of a tolerable intake

A number of effects produced by dioxins in experimental animals and observed in occupationally or accidentally exposed humans are clearly high dose effects and not directly relevant for the evaluation of dioxin exposure of the general population at background levels.

Many of the non-cancer effects observed in mainly adult male workers occupationally exposed to high levels of TCDD and higher chlorinated PCDDs were transient effects disappearing after the end of exposure. A few conditions appear to be in excess among highly exposed cohorts when compared to unexposed referent groups including alterations in lipids, fasting plasma glucose and GGT concentrations as well as mortality from cardiovascular disease. These effects were associated with mean body burdens at the time of last exposure ranging from 28-400 ng/kg. In Seveso residents (children and adults) acutely exposed to TCDD alone (resulting in median serum lipid TCDD concentrations of 500 ng/kg in the zone of highest exposure, zone A), a variety of transient effects were seen, including chloracne, increases in serum enzymes (GGT) and alterations in lymphocyte counts. Studies on children from zone A did not reveal effects on the immune competence. Mortality studies have indicated an excess of deaths due to cardiovascular diseases in males from zone A while there has also been reported an alteration in the sex ratio (excess females) of infants born to parents both resided in zone A.

Neurodevelopmental delays and neurobehavioural effects including neonatal hypotonia have been reported in studies of three large cohorts, two in the US and one in The Netherlands. However, the age at which the effects occurred and the tests used to detect them were not the same. Thyroid hormone levels were evaluated in two cohorts in The Netherlands with similar exposure to PCDDs/DFs and total PCBs. In utero exposure to total TEQs, as measured in mother's milk, may have influenced thyroid hormone status (TT4, TSH) in infants up to 3 months of age. Multiple, persistent effects occurred among highly exposed children who had transplacental exposure in Yusho and Yucheng. The effects included ectodermal defects, global persistent developmental delays, low birth-weight, mild persistent behaviour disorders, decrease in penile length at puberty, reduced height among girls at puberty and hearing loss.

Overall it should be noted that the observed changes in neurodevelopmental parameters and hormone levels in the non-accidentally exposed cohorts were within the normal range and considered without clinical relevance. The associations observed were due to prenatal (*in utero*) exposure rather than to postnatal exposure (mothers milk). Infants receiving breast feeding had higher scores in neurobehavioural tests than bottle-fed infants. However, this beneficial effect of breast-feeding diminished with higher contamination levels of PCBs, PCDDs and PCDFs in human milk.

The Committee found that the derivation of a tolerable intake useful for protection of the general population should be based on the most sensitive adverse effects observed in experimental humans or animals after TCDD exposure. In addition, a discussion of the carcinogenic properties of dioxins was considered appropriate because this is the effect most often cited by the news media, although it is not the most important adverse effect in relation to background dioxin exposure from food.

4.1. Carcinogenicity

Based on limited evidence in humans and sufficient evidence in experimental animals as well as mechanistic considerations the overall evaluation made by the International Agency for Research on Cancer (IARC, 1997) was that “TCDD is carcinogenic to humans (Group 1)”. In most studies, humans were exposed to mixtures of PCDDs including TCDD, as contaminants of phenoxy herbicides and chlorophenols. These epidemiological studies have involved subjects with the highest recorded exposures to TCDD. The epidemiological evidence obtained from the most highly TCDD-exposed cohorts studied produced the strongest evidence of increased risks for all cancers combined. The relative risk for all cancers combined in the most highly exposed and longer latency sub-cohorts was 1.4. In several of these studies excess risks were observed for soft tissue sarcoma and also for lung cancer, non-Hodgkin lymphoma and digestive tract cancers. Statistically significant excess risks were observed in individual cohorts for a variety of other cancer sites including multiple myeloma, oral cavity, kidney cancer, leukaemia and breast cancer in women. The equivalent lifetime doses of these populations were 2-3 orders of magnitude higher for TCDD, and 1-2 orders of magnitude higher for PCDD/PCDFs than those experienced by the general population (WHO, 1998).

In the long-term carcinogenicity study in rats by Kociba *et al.* (1978), in which an increase in liver tumours was observed, the LOAEL (10 ng/kg bw/day) corresponds to a steady state body burden of 294 ng TCDD/kg bw. In order for humans to obtain a similar steady state body burden, the kinetic model presented in Section 3 above predicts a human daily intake value of 150 pg/kg bw/day. Body burdens of 109-7000 ng/kg bw estimated from back-

calculated blood levels of TCDD in occupational cohorts, providing limited evidence of a human cancer response, overlap with the estimated body burden of 2976 ng TCDD/kg bw in the highest dose group (100 ng/kg bw/day) of the Kociba study (WHO, 1998).

Since the evaluations performed by IARC (IARC, 1997) and WHO (WHO, 1998) the hypothesis of a causal link between exposure to TCDD and occurrence of cancer in humans has received further support by new epidemiologic studies. Hoppin *et al.* (1998, 1999) have investigated the association between soft tissue sarcomas and occupational exposure to herbicides and chlorophenols, showing that malignant fibrohistiocytic sarcomas and leiomyosarcomas were most specifically concerned (see also comment by P. Vineis, 1999). Bertazzi (1999) extended up to 1996 the mortality follow-up in Seveso, showing an almost twofold statistically increased occurrence of cancer of the lymphatic and haematopoietic tissues in both sexes in the most contaminated areas (A + B). The study is continuing to try to overcome the existing limitations (few individual exposure data and small exposed population size for certain cancer types). In addition, molecular epidemiology studies have been initiated to clarify mechanisms of TCDD effects in man.

Steenland *et al.* (1999) have extended by 6 years the follow-up period of a U.S. cohort of 5172 workers, the largest of the four industrial cohorts considered by IARC (1997). In this study, where a job-matrix was developed, significant positive linear trends in SMRs with increasing exposure for all cancers combinened and for lung cancer were found. The SMR for all cancers combined for the highest group was 1.60 (95% confidence interval: 1.15-1.82). The results of this study suggest that high TCDD exposure results in an excess risk of all cancers combined, without any marked specificity. The excess cancer was limited to the highest exposures, that were likely to be 100-1000 times higher than those of the general population and similar to the doses used in experimental animals.

Recently, an apparent cluster of soft tissue sarcomas has been reported in the population resident in the proximity of an incinerator of industrial wastes in Mantua, Italy (Costani, 1998). An *ad hoc* epidemiologic study is now addressing the issue of an association between residence near the incinerator, exposure to dioxin, and risk of soft tissue sarcoma in Mantova.

The Committee considered the available experimental data and based on these and mechanistic information concluded that TCDD is a multi-site carcinogen in experimental animals, through a mechanism very likely involving the Ah receptor. TCDD is not a direct acting genotoxic agent but a tumour-promoting agent in experimental animals.

Several modes of action for the tumour promotive effect of TCDD have been hypothesised:

- binding to the Ah receptor mediates an increase in the expression of genes involved in cell growth and differentiation,
- induction of cytochrome P450 (CYP1A1 and CYP1A2 expression) mediates oxidative stress which leads to increased DNA-damage,
- inhibition of apoptosis favours expansion of pre-neoplastic cell populations.

The Ah receptor is a ubiquitous transcription factor in rodents and humans with two major functions: enhancement of transcription of a battery of genes and immediate activation of tyrosine kinases. The induction of CYP1A1 and CYP1A2 is the best-studied event in rodent and human cells; its role in carcinogenesis is unclear. Whereas CYP1A1 is induced in several organs and tissues, CYP1A2 seems to be induced only in the liver. Parallel increases in gluthathione transferase and glucuronidase activities seem also to occur appreciably only in

the liver. The widely diffused and powerful induction of CYP1A1 might explain the modest and variable increases of different types of tumours in several organs/tissues in man, whose incidence may be related to the variable total exposure to environmental carcinogens.

The Committee noted that while TCDD given prepubertally to female rats inhibited cell proliferation in the mammary gland, prenatal single exposure to TCDD lowered the mammary gland differentiation with a long-lasting effect resulting in more mammary tumours by DMBA at postpubertal age (Brown *et al.*, 1998).

5. Pivotal effects in experimental animals

In its assessment, the Committee focused on adverse effects seen at low TCDD doses. A number of biochemical changes, such as enzyme induction, enhanced expression of growth factors and enhanced oxidative stress have been noted in experimental animals at TCDD body burdens within a lower range of 3-10 ng/kg bw. The Committee did not find it appropriate to use these adaptive changes for the derivation of a tolerable daily intake of dioxins. While these effects are observed at the lowest body burdens, they are considered to be early markers of exposure to dioxins or events induced by dioxin-like compounds in animals and in humans that may or may not result in adverse effects.

The Committee also considered a study of enhanced viral sensitivity reported in mice following acute exposure to 10, 50 or 100 ng TCDD/kg bw (Burleson *et al.*, 1996) and noted the lack of a dose-response relationship in this study. Given the contradictory responses observed in immune parameters in general in experimental studies of TCDD and the concern raised about using these immune responses for extrapolation to very low doses, the study was not considered appropriate to include in the range of pivotal LOAELs. In addition, children from Seveso with chloracne, who had been exposed acutely to high doses of TCDD, exhibited only minor transient alterations in various non-specific immune system parameters.

The Committee identified the most sensitive adverse effects to be developmental and reproductive effects in rats and monkeys, along with the endometriosis found in monkeys. In the following section the Committee evaluates these studies and discusses the LOALs and the associated body burdens.

5.1. Effects on the reproductive organs in the offspring of TCDD-exposed rats

5.1.1. Studies used by WHO

Gray *et al.* (1997a) reported on the reproductive effects of TCDD in the male offspring of Long Evans Hooded rats. Although being part of the same study, the reproductive effects in the female offspring are reported in Gray *et al.* (1997b). The purpose of the study was to expand the dose response data for the reproductive effects in offspring seen in an earlier study by the same group that used only one dose level, i.e. 1 µg TCDD/kg b.w. (Gray *et al.*, 1995).

In this study pregnant Long Evans Hooded rats (12 per group) were administered 0 (vehicle control), 0.05, 0.20, or 0.80 µg TCDD/kg b.w. by gavage in corn oil on gestational day 15 (GD15), which marks the onset of the endocrine-sensitive phase of sexual differentiation. On

postnatal day 3 (PND3) the litters were standardised, where possible, to 5 males and 3 females (so as to minimise any variation in postnatal development due to litter size).

The growth of the offspring was recorded and the male pups were examined for eye opening and retained nipples/areola on PND13-16. Age and body weight at puberty (preputial separation) was noted from PND34 onwards. One male per litter (10-11 males per dose group) was killed at PND49 and PND63 and blood was collected for serum testosterone assay. Body, pituitary, adrenal, kidney, liver, ventral prostate, seminal vesicles, testes, and cauda and whole epididymides weights were recorded. From 9 months of age the remaining males (2-3 per litter) were allowed to mate and ejaculated sperm counts (ESC) measured from the uterine contents of the necropsied females. At 15 months the remaining male offspring were killed and organ and tissue weights recorded as on PND49 and 63. The ventral prostate and one epididymis were fixed for histological evaluation.

The percentage of male pups displaying eye opening at PND14 and 15 was significantly increased in all treatment groups. In the 0.80 µg/kg dose group, survival and, except at PND15, body weights of the pups were statistically significantly reduced. TCDD delayed puberty (indicated by preputial separation) in the high (by 2.6 days) and mid (1.2 days) dose groups ($p<0.01$). Glans penis weight was reduced in the mid and high dose groups at 15 months. However, there was no indication of antiandrogenic activity, i.e. retained nipples, urogenital malformations, or a reduction in serum testosterone (T) levels at PND49, PND63, and 15 months to suggest that this delay in puberty and reduction in glans penis weight is due to altered androgenic status. At PND49 there was a transient reduction in seminal vesicle and ventral prostate weights (testosterone-dependent tissues) in the high dose group.

Cauda epididymal sperm numbers (whole epididymis sperm numbers at PND49) were significantly reduced at PND49, PND63 and 15 months in the high dose group and also in the mid dose group at the latter time. At 15 months there was a non-significant increase in histologically-determined chronic inflammation of the epididymis at all dose levels. There were no effects on testis sperm numbers. At 15 months ESC were reduced in all dose groups (though the reduction was only significantly different from controls in the lower two doses when the data were pooled with the data from the earlier Gray *et al.*, 1995 study). However, there was no effect on mating behaviour, as indicated by the number of copulatory plugs.

The authors note that testis sperm number and testis weight were not affected at doses of TCDD that reduce epididymal and ejaculated sperm counts. They speculate that the reduction is due to effects on the epididymis itself, e.g. reduced storage, although, because the reduction of ESC was greater than epididymal sperm numbers, other factors e.g. subtle behavioural alterations, play a role in the reduced ESC. The authors discuss the accelerated eye opening and the non-significant reduction in ESC at 0.05 µg TCDD/kg b.w. as critical endpoints.

Gray *et al.* (1997b) is the accompanying paper to Gray *et al.* (1997a), reporting the reproductive effects in female offspring of mothers (12 per dose group) administered 0 (vehicle control), 0.05, 0.20, or 0.80 µg TCDD/kg b.w. by gavage in corn oil on gestational day 15 (GD15), hereafter referred to as study 1. The paper also reports on two parallel studies designed to determine if specific effects on the reproductive system in the offspring i.e. vaginal threads, are mediated *via in utero* and/or lactational TCDD exposure (study 2), and to determine potential effects on ovarian and uterine function (study 3).

In study 1, the offspring were examined daily for partial and complete vaginal opening and the recording of external reproductive malformations. One female per litter was killed on

postnatal day 70 (PND70) and body, brain, pituitary, adrenals, liver, kidneys, ovaries and the entire reproductive tract (uterus, cervix and vagina) weights were recorded. Ovary, uterus and vagina were fixed for histological evaluation. Remaining females (2 per litter) were placed on continuous mating from PND100. At 20 months they were necropsied and subject to same histological evaluation as PND70.

TCDD significantly delayed onset of puberty, i.e. vaginal opening, in the high dose group (non-significant delay in mid dose group) without effect on body weight. There were dose-related increases in the percentage of female offspring with either temporary or permanent vaginal threads (significant at mid and high dose groups). There was significant clefting of the phallus at the high dose. In the mid and high dose groups the size of the urethral slit (US) and the distance from the tip of the phallus to the urethral opening (UP) were significantly increased while the distance from the urethral opening to vaginal caudal opening (UV) was significantly decreased. This represents a mild form of hypospadias, though the urethral opening was always separate from the vaginal orifice. There was a marginal non-significant reduction in ovarian and reproductive tract weights at PND70 in the high dose group though there were no associated histological abnormalities. In the remaining female offspring the fertility rate was unaffected (as assessed by number of pups/litter and number of litters over the 10 month period), though time to first pregnancy was significantly delayed in the high dose group.

Histological examination of the tissues from the ageing female rats revealed some differences between control and treated groups. However, there was no clear evidence of a dose-response relationship except in regard to diffuse squamous hyperplasia of the cervix and hyperkeratosis of the vagina.

Study 2 was designed to define the influence of pre- and postnatal exposure to TCDD on the development of abnormalities of the reproductive tract. An unspecified number of pregnant rats were administered either 0 or 1 µg TCDD/kg b.w. by gavage in corn oil on GD15. At parturition, 5 control and 5 treated litters, with at least 4 female offspring per litter, were selected and all pups were cross-fostered to generate control, *in utero*, lactational, and *in utero* + lactational exposure groups. Cross-fostering was initially within the treatment group but, on an unspecified day, further exchange was made between treated and control dams. Litter sizes were maintained at eight pups by retaining male offspring.

In this study the controls and lactationally-exposed female offspring did not develop vaginal threads; these occurred only in the female offspring with *in utero* TCDD exposure. At necropsy at PND80 *in utero* exposed offspring had increased incidence of cleft phallus and altered US, UP and UV measurements. None of these effects were seen in the lactationally-exposed group.

In study 3, pregnant rats were dosed with either 0 or 1 µg TCDD/kg b.w. (n = 7 or 5 respectively) by gavage in corn oil on GD15. After birth, litters were adjusted to eight pups (with seven female pups per litter where possible).

One female per litter was necropsied at PND 21 and 28 and serum and ovarian oestrogen levels determined. At PND 28, one female per litter was injected s.c. with oestradiol benzoate to stimulate uterine growth and uterine growth determined 24 hours later. Remaining female offspring were necropsied at PND80 and ovaries and reproductive tract subject to histological evaluation (no results of this evaluation were reported).

Serum and ovarian oestrogen levels were unaffected. Uterine growth in response to oestradiol benzoate was unaffected.

The authors mention a parallel study in Long Evans rats using the same dosing regime (0.05, 0,2, 0,8, or 1,0 µg ^{3}H-TCDD/kg bw by gavage on GD15) to determine the maternal and foetal distribution of orally administered TCDD (Hurst *et al.*, 1997, abstract only). A full report has been published recently (Hurst *et al.*, 2000). In this study, TCDD levels were measured in maternal and foetal tissues at GD16 and GD21. For all doses at GD16, the greatest amount of radioactivity in the dam was found in the liver (34, 30, 47, and 39% of the dose), followed by adipose tissue (11, 6, 7, and 8% of the dose). At GD21 the liver still contained large amounts of TCDD (15, 16, 23, and 27% of the dose) especially at the higher doses although more had redistributed to the adipose tissue (37, 20, 19, and 16% of the dose), in particular at the lowest dose. Maternal body burdens on GD16 were calculated to be 30.6 (60% of the dose), 97,4 (48% of the dose), 522.8 (65% of the dose), and 585.2 ng TCDD/kg bw (59% of the dose), respectively. On GD21 the maternal body burdens had decreased to 26.6, 76.2, 327.8, and 431.1 ng TCDD/kg bw. The foetal concentrations of TCDD were 5.3, 13.2, 39.1, and 55.7 pg/g wet weight on GD16 and 4.3, 14.6, 32.2, and 36.4 pg/g on GD21. The maternal blood concentrations on GD16 were determined to be 2.2, 7.9, 26.7, and 50.9 pg TCDD/g compared to 1.1, 2.1, 15.6, and 23.0 pg/g on GD21. The authors examined the relation between the foetal concentration of TCDD and the magnitude of responses observed in the parallel studies by Gray and co-workers mentioned above. They found that for the responses studied (delayed puberty and decreased epididymal sperm counts in male offspring, and malformations of external genitalia of female offspring), the foetal concentration of TCDD at GD16 adequately predicted the intensity of the responses. In addition, there was a strong correlation between foetal body burden, maternal body burden and maternal blood levels at GD16. Hurst *et al*., (2000) also reported that a foetal concentration of 16 pg TCDD/g found on GD16 following a gavage dose of 1.15 µg/kg bw on GD8 adequately predicted the severity of the effects reported after a single bolus dose of 1 µg/kg bw administered on GD8 (Gray *et al*., 1995; Gray and Ostby, 1995). The magnitude of the responses were similar to those seen after a single bolus dose of 0.2 µg 2,3,7,8-TCDD/kg bw administered on GD15.

Conclusions on the studies by Gray *et al*. (1997a, 1997b)

In rat male offspring, *in utero* TCDD exposure produced accelerated eye opening and a non-significant reduction (25%) in ejaculated sperm count at 0.05 µg TCDD/kg b.w. These effects were not due to altered androgenic status.

In rat female offspring, *in utero*, though not lactational, TCDD exposure produced statistically significant genital malformations, i.e. vaginal threads and alterations of the external genitalia, at 0.2 µg TCDD/kg b.w. These effects are not a consequence of reduced perinatal ovarian or serum oestrogen levels.

Comments

The authors note that the lowest dose of 0.05 µg TCDD/kg body weight was associated with a maternal tissue concentration that exceeds the average human tissue concentration (estimated at 13 ng/kg bw) by only about 3-fold. The implication is that foetal animals in this study were exposed to TCDD levels only 3-fold higher than human foetal exposure. However, there are a number of unsubstantiated assumptions to this hypothesis, namely:

- toxicokinetics of animal and human placental transfer may differ (though this may increase or decrease human foetal exposure relative to foetal rats),

- bioavailability of TCDD to foetus at a given maternal body burden may differ between a bolus dose (as in this study) and dietary exposure at steady state. Intuitively differences in bioavailability would seem likely. Following a bolus administration, serum TCDD levels would be elevated before redistribution to the tissue compartments. It is likely that TCDD would still be redistributing to the fat compartment when concentrations were measured only 24 hours following oral administration. In contrast, low-level chronic exposure will not significantly elevate serum levels. 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. Hence a more appropriate surrogate for foetal exposure would be the peak maternal serum TCDD concentrations.

GD15 marks the onset of the endocrine-sensitive phase of sexual differentiation in rats and therefore represents a critical window for foetal exposure for these reproductive endpoints. In fact, a dose of 1.0 µg TCDD/kg bw given on GD8 produced responses similar to a dose of 0.2 µg TCDD/kg bw given on GD15. In addition, the foetal concentrations of TCDD measured on GD16 were reported to be very similar (Hurst *et al.*, 2000). This would suggest that the critical determinant of these reproductive effects is the foetal dose on GD15, which, as noted above, is likely to be higher following a single bolus dose on this day than that resulting from lower level chronic exposure.

The study by Hurst *et al.* (2000) indicate that the absorption rate of TCCD given by a bolus dose to pregnant rats was about 60%.

5.1.2. Additional studies

In fact, the first studies to report on the effects of prenatal exposure to dioxins in rats were published by Peterson and co-workers in 1992 in a series of three papers (Mably *et al.*, 1992a, b, c, reviewed by Peterson *et al.*, 1993). Initially, they treated pregnant Holzman rats with 1.0 µg TCDD/kg bw on Day 15 of gestation and found plasma testosterone concentrations in fetal males to be significantly reduced on GD18 through 21. The surge in plasma testosterone concentrations shortly after birth was also significantly reduced, as was anogenital distance, an androgen-dependent parameter. To further investigate the effects of perinatal TCDD exposure on the male reproductive system, rats born to dams given bolus doses of TCDD (0.064, 0.16, 0.40, or 1.0 µg/kg bw) on GD15 were evaluated from birth through sexual maturation. There was little evidence that TCDD caused maternal toxicity. Signs of overt toxicity in offspring were limited to an 8% reduction in live births (highest dose only) and to decreases in body weight gain and feed consumption (two highest doses only) which disappeared by early adulthood. With respect to androgenic status, maternal TCDD doses as low as 0.16 micrograms/kg produced significant dose-related decreases in the anogenital distance at birth, delays in testicular descent, and decreases in seminal vesicle and ventral prostate weights (Mably *et al.*, 1992a).

In the second paper (Mably *et al.*, 1992b) masculine sexual behaviour at approximately 60, 75, and 115 days of age was reported. When TCDD-exposed males were caged with receptive control females their mount, intromission, and ejaculation latencies were far longer than normal. These effects were dose-related and were statistically significant at maternal doses as low as 0.16, 0.064, and 0.16 µg TCDD/kg bw, respectively. The numbers of mounts and intromissions to ejaculation were slightly increased by TCDD, while copulatory rates [(mounts + intromissions)/min] were significantly decreased at the three highest maternal doses. Except for a modest increase at the higher doses, TCDD had little effect on the

postejaculatory interval. Following assessment of their masculine sexual behavior, the males were castrated and 6 weeks later tested for feminine sexual behavior (lordosis). After being primed with oestradiol benzoate and treated with progesterone, males displayed dose-related increases in lordosis quotient and lordosis intensity in response to being mounted by another male. These effects were statistically significant at maternal doses as low as 0.16 and 0.40 μg TCDD/kg, respectively. To determine if perinatal TCDD exposure alters the sexually dimorphic regulation of luteinising hormone (LH) secretion, the LH secretory responsiveness of the hypothalamic/pituitary axis to ovarian steroids was assessed. In unexposed, gonadectomised female rats primed with oestradiol benzoate, progesterone injection produced a surge in plasma LH concentrations, whereas in similarly treated control males, plasma LH concentrations were unaffected by progesterone. In castrated, oestradiol benzoate-primed male rats that were perinatally exposed to TCDD, progesterone treatment produced dose-related increases in plasma LH concentrations that were statistically significant at the two highest maternal doses. The authors conclude that in utero and lactational exposure to small amounts of TCDD demasculinises and feminises male rats. These effects could not be accounted for by TCDD-induced hypophagia, modest reductions in adult plasma androgen concentrations, possible nonspecific changes in motor activity, or possible reductions in penile sensitivity to sexual stimulation. The altered sexual behaviors and LH secretion were observed when nearly all TCDD had been excreted (as evidenced by uninduced hepatic ethoxyresorufin-O-deethylase activity). The authors suggest that *in utero* and lactational exposure to TCDD impairs sexual differentiation of the CNS.

In the third paper (Mably *et al.*, 1992c) the effects on spermatogenesis and male reproductive capability were reported. Testis, epididymis, and cauda epididymis weights were decreased in a dose-related fashion at 32, 49, 63, and 120 days of age, when males were at the juvenile, pubertal, postpubertal, and mature stages of sexual development, respectively. When measured on Days 49, 63, and 120, daily sperm production by the testis was reduced at the highest maternal TCDD dose to 57-74% of the control rate. Cauda epididymal sperm reserves in 63- and 120-day-old males were decreased to as low as 25 and 44%, respectively, of control values, although the motility and morphology of these sperm appeared to be unaffected. The magnitude of the effects tended to lessen with time; nevertheless, the decreases in epididymis and cauda epididymis weights, daily sperm production, and cauda epididymal sperm number were statistically significant at the lowest maternal dose tested (0.064 micrograms TCDD/kg) on Day 120 and at most earlier times. To determine if in utero and lactational TCDD exposure also affects male reproductive capability, rats were mated at approximately 70 and 120 days of age with control females. Little if any effect on fertility was seen, and the survival and growth of offspring was unaffected. These results are not inconsistent with the pronounced reductions in daily sperm production and cauda epididymal sperm reserves caused by perinatal TCDD exposure since rats produce and ejaculate far more sperm than are required for normal fertility. The TCDD-induced reduction in spermatogenesis could not be accounted for by concurrent effects on plasma follicle-stimulating hormone or androgen concentrations or by undernutrition. Leptotene spermatocyte to Sertoli cell ratios were not affected in 49-, 63-, and 120-day old rats. This suggests that the decrease in spermatogenesis was caused by impaired division and/or increased attrition of cells during the conversion of leptotene spermatocytes to spermatozoa and/or by a reduction in Sertoli cell number.

Subsequent studies were not been able to repeat the effects that were observed on circulation male hormone levels (Gray *et al.*, 1995). In these studies pregnant Long-Evans rats were treated on GD15 with a single dose of 1 μg TCDD/kg bw. No difference in latency to mount

was observed, but more mounts were necessary for ejaculation. Also, no effect was seen on female sex behaviour (lordosis) in castrated male Long-Evans rats. However, anogenital distance was reduced in male pubs at birth, testicular sperm production was down 30% at puberty, but in adulthood was down only 5% and epididymal sperm count were decreased 59% at puberty and 30% at adulthood. Ejaculated sperm count, measured at mating was reduced by 60%. Gray *et al.* (1995) found similar, but weaker effects in Syrian golden hamsters treated with 2 μg TCDD/kg bw at GD11.

Many of the effects on the developing reproductive organs reported by Mably *et al*. (1992c) and Gray *et al.* (1995, 1997a, b) have been repeated by others in male and female offspring of Holzman, Long-Evans, and Wistar rats (Flaws *et al.*, 1997; Roman and Peterson, 1998; Roman *et al.*, 1998; Faqi *et al.*, 1998; Loeffler and Peterson, 1999). In all these studies bolus doses of 0.25 or 1 μg TCDD/kg bw, given on GD15, were used. From these studies it also appear that the Holzman rat used by Mably *et al.* is more sensitive than the Long-Evans rat used by Gray *et al.*

5.1.3. Conclusion

There is sufficient evidence that prenatal TCDD exposure of rodents produce a number of adverse effects on the developing male and female reproductive organs and their functions. The Committee agrees with WHO that 0.2 μg TCDD/kg bw administered by gavage as a single bolus dose to pregnant rats at GD15 represents a LOAEL (and 0.05 μg TCDD/kg bw a NOAEL) for induction of vaginal threads and alterations of the external genitalia in the female offspring. In the male offspring, 0.05-0.064 μg TCDD/kg bw represents a sensitive LOAEL in producing accelerated eye opening and a reduction in ejaculated sperm count. A clear effect on this and several other parameter of male reproductive function was seen at a dose level of 0.16 μg TCDD/kg bw.

It is not clearly documented how WHO arrived at a body burden of 28 ng TCDD/kg bw for the LOAEL for decreased sperm count in the male offspring as presented in Table 4 of the WHO executive summary. References are made to the studies by Gray *et al.*, (1997a) and Mably et al (1992c) in which the doses of TCDD used were 50 ng/kg bw and 64 ng/kg bw, respectively. Using an absorption rate of 60% as found by Hurst *et al.* (2000) would predict body burdens of 30 and 38 ng/kg bw, respectively.

Similarly, it is not clearly documented how WHO arrived at a body burden of 73 ng TCDD/kg bw for the LOAEL of 0.2 μg TCDD/kg bw for increased genital malformations in female offspring reported in the paper of Gray *et al.* (1997b). Using the assumption of 60% absorption would produce a LOAEL body burden of 120 ng TCDD/kg bw for malformations in female offspring.

5.2. Effects on development of endometriosis in rhesus monkeys

5.2.1. Study used by WHO

This study by Rier *et al.* (1993), initially undertaken in 1977 to investigate long-term reproductive effects of TCDD exposure in rhesus monkeys, was adapted to investigate the dose-response of the incidence/severity of endometriosis when one animal died during the study. The death was attributed to severe endometriosis (which can only be diagnosed by direct visualisation of the peritoneal cavity).

At the onset of the original study, 24 female monkeys, 6-10 years old, were administered 0, 5, or 25 ppt TCDD in the diet (8 animals per group). Bowman *et al.* (1989) reported that the low dose group was exposed for 3.5 years and the high dose group for 4 years. Following cessation of exposure the monkeys were observed for a further 10 years. During the observation period, four animals in the high dose group died (three as a result of severe endometriosis, the other from a fight), one monkey died in the low dose group and two in the control group (all three without signs of endometriosis). At end of the observation period the 17 live monkeys were diagnosed for endometriosis by laparoscopy, the presence and severity of which was determined according to human criteria. The three monkeys that died of extensive endometriosis in the high dose group were included in the count of animals affected by endometriosis (the four monkeys that died of other causes were not so considered).

The authors report that the prevalence of spontaneous endometriosis in monkeys housed in the colony (determined from 304 autopsy records) is age-associated and is not seen in animals <13 years old. In animals >13 years of age there is a 30% prevalence. All the animals evaluated in this study (including the 3 that died) were older than 13 years. Endometriosis was diagnosed in 33% of the controls (2 of 6), 71% of the low dose group (5 of 7) and 86% of the high dose group (6 of 7). The increased incidence in the high dose group was statistically significant. Moderate to severe endometriosis (stages II, III and IV) was not seen in control animals, but was diagnosed in 43% (3 of 7) of the low dose group and 71% (5 of 7) of the high dose group. Linear regression analysis of the severity of the disease was correlated with the cumulative dose administered.

Reproductive function (i.e. ability to conceive, no. of live births, spontaneous abortions) was assessed during the actual TCDD exposure period, i.e. approximately the initial four years. Reproductive function in the low dose group was not affected but was impaired in the high dose group.

Conclusion on the study by Rier *et al*. (1993)

The incidence and severity of endometriosis in rhesus monkeys fed diets containing 0, 5, or 25 ppt TCDD was correlated with the cumulative TCDD exposure. Thus, chronic administration of 5 ppt in the diet represents a LOAEL for this effect in the female rhesus monkey.

In support of the conclusion, growth of surgically induced endometriotic cysts has been promoted in rats and mice following exposure to TCDD (and 2,3,4,7,8-PCDF), although at much higher doses than in the monkey (Cummings *et al.*, 1996, Johnson *et al.*, 1997).

Comments

Endometriosis occurs exclusively in menstruating species, e.g. humans and nonhuman primates. In monkeys the disease models human disease anatomically and clinically, though it is reported to be potentially fatal in monkeys. The incidence of spontaneous endometriosis in the colony is very high (approximately 30%), therefore whether this is an appropriate model for humans (where the prevalence is reported to be 10% in reproductive-aged women) is uncertain. Also, since the onset of endometriosis in the animals occurred some years after exposure ended it is important to know whether the mothers of the control group underwent repeated lapararoscopy for the removal of mesenteric fat samples. If they did not this may represent a confounding factor in the development of endometriosis. The sample number is small, i.e. 20 animals in total. However, the results for incidence of endometriosis in the groups fed TCDD were statistically significant.

A paper by Bowman *et al.* (1989) have reported on the half-life and maternal-to-infant transfer of TCDD in these monkeys. During the period of exposure the females delivered two birth cohorts: cohort 1 at 16 months and cohort 2 at 36 months. Cohort 3 (3 of the 7 surviving females in the high dose group were not bred) was delivered at 18 months post exposure i.e. approximately 5.5 years after start of the study. Fat biopsies (not reported whether subcutaneous or mesenteric fat) were taken from the 3 females that were not bred for cohort 3 at 0, 6 months and every 3 months thereafter, where 0 refers to the time at which exposure ceased. The fat of those females that were bred has also been sampled to determine the half-life of TCDD in the animals. Subcutaneous or mesenteric fat was sampled from the mothers at parturition and weaning, from the offspring at weaning and from any non-pregnant mothers at the same calendar time. Milk was sampled on all mothers nursing cohort III, once per week for the first 6 weeks and then biweekly. Selected milk and fat samples were analysed for TCDD. The results, as presented in this paper, are very difficult to interpret. Apparently at the time of going to press not all samples had been analysed. However, it is reported that at the birth of cohort 3 (or presumably the same calendar time) the mean fat TCDD concentration (the average of subcutaneous or mesenteric fat) in 8 low dose and 7 high dose females was 54 and 219 pg/g respectively.

Tissue concentrations were only determined 18 months after cessation of TCDD exposure and would therefore have declined according to the half-life, estimated as 391 days. Furthermore, prior to sampling the females had given birth between 1-3 times – as a result a fraction of the TCDD body burden would have been lost *via* lactation. Therefore, it is unlikely that the measured tissue concentrations truly represent the steady state tissue concentrations that would have been attained during the exposure period, and they do not represent an appropriate measure of, or are likely to substantially underestimate, tissue concentrations associated with the chronic intake of TCDD with diets containing 5 or 25 ppt TCDD.

In the paper of Bowman *et al.* (1989) it is reported that animals consumed 200 g of chow/day. However, the weights of the monkeys were not reported. Therefore, estimates of the daily intake and body burdens of TCDD by these monkeys would depend on assumption on their body weights. A default assumption would be that monkeys weigh 5 kg and thus the dietary levels are equivalent to 0, 0.2, and 1 ng TCDD/kg bw/day. However, for the low dose 5 ppt group daily intakes of 0.151 and 0.126 ng TCDD/kg have been reported by DeVito et al. (1995) and The Health Council of The Netherlands (1996), respectively. The figure used by WHO was 0.16 ng TCDD/kg bw/day. In all these cases, the intake estimates were not adequately documented.

WHO state that the body burden of the monkeys in the 5 ppt group at the end of exposure was 42 ng/kg bw. This figure apparently originates from DeVito *et al.* (1995), who calculated the body burden in the mothers who after 16.2 months of exposure gave birth to pups that underwent behavioural testing (see later). However, DeVito *et al.* (1995) estimated the body burden at the end of exposure (after 4 years) at 69 ng/kg bw. In the calculations done by DeVito *et al.* (1995) it was assumed that 86% of the dietary TCDD was absorbed and the half-life for whole body elimination of TCDD was 400 days. If it is assumed that only 50% of dietary TCDD is absorbed, the daily intake of 0.151 ng/kg bw stated by DeVito *et al.* (1995) would produce a body burden of 39 ng/kg bw after 42 months.

For the range of daily intakes reported (0.126-0.2 ng TCDD/kg bw/day) the assumption of 50% absorption would produce a range of body burdens of 31-51 ng/kg bw.

5.2.2. Conclusion

Female rhesus monkeys fed a diet containing 5 or 25 ppt 2,3,7,8-TCDD 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 2,3,7,8-TCDD exposure. The severity of the disease was correlated with the cumulative 2,3,7,8-TCDD exposure and the increased incidence in the high dose group was statistically significant (Rier *et al.*, 1993). Thus, chronic administration of 5 ppt 2,3,7,8-TCDD of diet represents a sensitive LOAEL for endometriosis in the female rhesus monkey.

The Committee noted that there were conflicting estimates of the maternal daily intake of TCDD related to the findings in this monkey study and that these would predict a steady state body burden at the end of the exposure period within the range of 31-51 ng/kg bw, assuming 50% absorption.

5.3. Effects on the learning behaviour of the offspring of TCDD-dosed rhesus monkeys

5.3.1. Study used by WHO

Schantz & Bowman (1989) assessed the potential effects of perinatal TCDD exposure on learning behaviour. It is part of a study investigating long-term reproductive effects of TCDD in rhesus monkey; the offspring assessed for behavioural effects were those of the monkeys investigated for endometriosis (Rier *et al.*, 1993).

Twenty-four female rhesus monkeys, 8 per group, were administered 0, 5, or 25 ppt TCDD in the diet. Eight low dose females and seven control females were bred with six males (some of which had previously been exposed to PCBs – Bowman *et al.*, 1989), delivering birth cohort 1 at 16 months. Six of the eight treated females delivered viable offspring and seven of the seven control females delivered viable offspring. Cohort 2 was delivered at 36 months after seven low dose females and five control females bred with the same six males (in both cohorts some offspring shared the same father). Five of the seven low dose females delivered viable offspring and three of the five control females delivered viable offspring. While the high dose group was allowed to mate they delivered too few viable offspring and were therefore not included within this study. The offspring exposure ceased at weaning at 4 months. Mesenteric fat was taken from offspring at 5, 12, 18 and 24 months, though at the time of going to press only the 5-month sample had been analysed for TCDD.

The offspring (as one exposed monkey refused to co-operate there were 10 exposed and 10 control monkeys) were subject to 4 discrimination reversal learning (DRL) tests at age of 14 months, i.e. spatial, spatial discrimination with irrelevant cues, colour, and shape DRL tests (in this order), and a delayed spatial alternation (DSA) test at 20 months.

There were no significant group mean differences in trials to criterion (defined as 9 correct out of 10 consecutive trials) on the spatial, spatial discrimination with irrelevant cues, and colour DRL tests. There were also no significant differences in trials to criterion on over-training (over-training is defined as 90% or more correct choices on 2 consecutive days) for these 3 tests. Trials to criterion decreased similarly across reversals for these 3 tests. There was a non-significant difference in trials to criterion for the acquisition for the shape DRL test i.e. 32 *versus* 15 trials. There was no difference on over-training. However, there was a significant difference in trials to criterion on the first reversal after acquisition for the shape

DRL test, i.e. 47 *versus* 27 trials. However, after the first reversal, trials to criterion decreased similarly across subsequent reversals (7 in total) in the treated and control groups.

While there were no group differences on the two spatial and colour DRL tests, regression analysis for TCDD exposed animals only showed that trials to criterion on the first reversal for the 2 spatial tests (though only the spatial DRL test appears to be a significant correlation) was positively correlated with TCDD fat concentration at 5 months (control fat concentrations were below the LOD and therefore were not included in the regression analysis). In contrast, there was a significant negative correlation between trials to criterion on the first reversal for the colour DRL test and TCDD body fat concentration. Performance on the shape DRL test (i.e. the only DRL with a group difference) was not correlated with TCDD body fat concentration.

There were no significant group differences on percentage correct performance or latency to respond on the DSA task, and neither performance was correlated with TCDD body fat concentration.

Conclusion on the study by Schantz & Bowman (1989)

Chronic exposure of female rhesus monkeys at 5 ppt TCDD in the diet produced a subtle change in cognitive recognition (object learning) of offspring delivered after 16.2 or 36.3 months. The change was correlated with the TCDD fat concentration in the offspring at 5 months of age.

Comments

It is noteworthy that only one significant TCDD related, possibly deleterious, difference between the groups was found namely deficits in object learning. In the same monkeys improvements in spatial learning was observed. The clinical significance of these findings remains unresolved. It may also be worth noting that the same group has undertaken studies on the offspring of monkeys fed Aroclor 1016 and 1248 in the diet. Performance in a similar shape DRL test was facilitated in the offspring born of mothers three years after their dietary exposure to 2.5 ppm of Aroclor 1248 had ceased (Schantz *et al.*, 1989).

The authors claim that the sensitivity of the DRL model is well established; impaired performance on the DRL occurs in the absence of any impairment on the DSA task. TCDD exposed offspring were significantly retarded on the first reversal of the shape DRL test (which the authors claim resembles first reversal learning deficits in lead-exposed monkeys) though were not impaired on acquisition of this test. Because reversals differ from acquisition in that the animal must inhibit a previously learned response (i.e. acquisition) in addition to learning a new one, the authors concluded that TCDD might interfere with the maturation of central inhibitory competence. Furthermore, the authors conclude that this is a cognitive deficit rather than an impairment of sensory or motor function as there was no impairment on the other 3 DRL tests and on the subsequent 7 reversals of the shape DRL test. Any impairment in the ability to discriminate stimuli (sensory) or to make the necessary motor response would have affected performance across all stages of all the DRL tasks. The authors state that the negative correlation between TCDD fat concentrations and performance on the colour DRL test is difficult to interpret and indicates the complexity of cognitive behaviour. The authors suggest that one possible mechanism by which TCDD may cause these “subtle cognitive” behavioural effects is *via* altered thyroid function (presumably hypothyroidism).

WHO state that the maternal body burden of the monkeys dams in the 5 ppt group was 42 ng/kg bw. This figure apparently originates from DeVito *et al.* (1995), who calculated the body burden in the mothers who after 16.2 months of exposure gave birth to pups that underwent behavioural testing. In the calculations done by DeVito *et al.* (1995) it was assumed that 86% of the dietary TCDD was absorbed and the half-life for whole body elimination of TCDD was 400 days. If it is assumed that only 50% of dietary TCDD is absorbed, the daily intake of 0.151 ng/kg bw stated by DeVito *et al.* (1995) would produce a maternal body burden of approximately 25 ng/kg bw after 16.2 months and of approximately 37 ng/kg bw after 36.3 months.

For the range of daily intakes by these monkeys estimated by different authors (0.126-0.2 ng TCDD/kg bw/day) the WHO procedure would produce a range of body burdens of 21-33 ng/kg bw at 16.2 months.

5.3.2. Additional relevant information

Spatial learning and memory was assessed in rats by Schantz *et al.* (1996) following *in utero* and lactational exposure to coplanar PCBs or TCDD. Pregnant Sprague-Dawley rats were dosed with PCB 77 (3,3',4,4'-tetrachlorobiphenyl), 2 or 8 mg/kg bw/day; PCB 126 (3,3',4,4',5-pentachlorobiphenyl), 0.25 or 1.0 μg/kg bw/day; TCDD, 0.025 or 0.1 μg/kg bw/day; or corn oil vehicle *via* gavage on gestation days 10-16. Litters were culled to eight on day 2 and weaned on day 21. Beginning on day 80, one male and one female from each litter were tested on an eight-arm radial maze working memory task. The TCDD-exposed rats displayed pronounced decreases in errors relative to controls. PCB 77- and PCB 126-exposed rats showed similar, but less pronounced, decreases in errors. The same animals were later tested on a T-maze DSA task, but no differences among groups were observed. The authors conclude that perinatal exposure to low doses of TCDD or structurally related coplanar PCBs appeared to facilitate acquisition of a working memory task on the radial arm maze. Earlier studies had shown that rats exposed to *ortho*-substituted PCBs did not differ from controls on the radial arm maze and were impaired on the T-maze DSA task (Schantz *et al.*, 1996). These findings suggest that coplanar and *ortho*-substituted PCBs may have different mechanisms of action on the CNS.

5.3.3. Conclusion

The Committee noted that subtle deficits were reported for one, among several, parameters related to cognitive recognition (object learning) in one study of offspring of rhesus monkeys chronically exposed to TCDD. The Committee was not able to determine the clinical significance for humans, if any, of this finding. Studies of offspring of rats exposed to TCDD and epidemiological studies of infants and children, in which the mothers were exposed to elevated concentrations of complex mixtures of environmentally persistent chemicals, including PCBs and dioxins, have produced contradictory findings. The Committee also noted the conflicting estimates of the maternal daily intake of TCDD related to the findings in the monkey study. However, all estimates predicted body burdens within the range of estimated TCDD body burdens for which other sensitive effects have been documented.

5.4. Effects on the immune system of the offspring of TCDD-dosed rats

The paper by Gehrs *et al.* (1997) describes a suppressed delayed type hypersensitivity (DTH) response to bovine serum albumin in the 4-5 month old offspring of F344 rat dams that

received 1 or 3 µg TCDD/kg bw by oral gavage on gestational day 14 (GD14). It forms the basis for a subsequent study on the dose-response effects of TCDD (Gehrs and Smialowicz 1998 (abstract only), 1999 (full paper)). The paper reports on two separate studies. In study 1, 13 pregnant rats were administered. While controls were used no further details are reported. At weaning, i.e. 4 weeks after birth, there were 19 male and 19 female treated offspring alive. At 14-17 weeks the offspring were subjected to three functional immune tests, i.e. splenic lymphoproliferative (LP) response, plaque forming cell (PFC) assay to SRBCs, and delayed type hypersensitivity (DTH) assay. The DTH response was determined seven days after a sensitising dose of bovine serum albumin (BSA). The rats were challenged by injection of heat aggregated BSA into the left footpad and saline into the right footpad and the DHT response was measured as the difference in thickness between the left and right hind footpads. Liver, spleen and thymus weights were recorded. Splenic cellularity and splenic phenotyping ($CD3^+/CD4^+CD8^+$; $CD3^+/CD4^-CD8^-$; $CD3^+/CD4^+CD8^-$; $CD3^+/CD4^-CD8^+$; splenic B-cells identified using antibodies to surface IgM) were also determined.

In study 2, pregnant rats were assigned to four groups: two of the groups were administered 1 µg TCDD/kg bw by oral gavage on GD14 and there were two control groups. On postnatal day 1 (PND1) cross-fostering of the offspring produced 4 distinct exposure groups, i.e. controls (group 1), *in utero* exposure only (group 2), lactational exposure only (group 3), and *in utero* and lactational exposure combined (group 4). Litters were then normalised to four males and four females per group. At weeks 1, 2, and 3, liver, spleen and thymus weight was determined for one animal of each sex from each litter. Splenic and thymic cellularity and phenotype were also determined. At five months the remaining male in each litter was subjected to the DTH test (there were insufficient controls to assess the females).

In study 1 the spleen weight was significantly increased in both sexes of non-immunised and SRBC-immunised rats though splenic cellularity was unaffected. In non-immunised rats, thymus weight was significantly decreased in the males only. In immunised rats thymus weight was significantly decreased in both sexes. The only significant splenic phenotypic alteration, which occurred in both sexes of immunised and non-immunised rats, was a decreased percentage of $CD3^+/CD4^-CD8^-$ cells. The splenic LP response to T-cell mitogens PHA and ConA, and the B-cell mitogen of S*almonella typhimurium* (STM) was unaffected in both sexes though in females the mitogenic response to the B and T-cell mitogen pokeweed (PWM) was significantly suppressed. While there was no significant suppression of the PFC assay, the DTH response was significantly suppressed in both sexes.

In study 2, week 1, three of the four thymic $CD3^+$ subsets were significantly altered ($CD4^+CD8^-$ subset was unaffected) in group 4. The $CD3^+/CD4^-CD8^-$ subset was also decreased in groups 2 and 3 for males and group 3 for females. While thymic cellularity was unaffected in males, it was significantly reduced in group 2 and 3 females. There were no significant effects on $CD3^+$ splenocytes and B-cell splenocytes. At week 2, only data for 4 are reported, though the authors state that intermediate effect levels were seen in groups 2 and 3. In group 4 there was a significant decrease in thymic cellularity in males (and a non-significant 30% decrease in females). Both sexes exhibited non-significant thymic atrophy. Thymic $CD3^+/CD4^-CD8^-$ and $CD4^+CD8^+$ subsets were decreased in both sexes. Splenocytes were unaffected. For week 3 only data for group 4 are presented (though again it is reported intermediate effect levels were seen in the other exposure groups). The percentage of thymic $CD3^+/CD4^-CD8^-$ and $CD4^-CD8^+$ subsets were altered. Thymic cellularity was decreased in both sexes, though only significantly in females. There was also non-significant thymic atrophy in both sexes. Splenocytes were unaffected. In 5-month old males the DTH response was suppressed in all three exposure groups, though only significantly in group 4.

In order to determine the lowest maternal dose of TCDD that produced DTH suppression in F344 rats, Gehrs and Smialowicz (1998, 1999) measured the DTH response to BSA in the 4 and 14 months old offspring of dams exposed by gavage to 0, 0.1, 0.3, or 1 μg TCDD/kg bw on GD14. In the males, modest but significant suppression was observed at a dose of 0.1 μg TCDD/kg bw at 14 months of age, while a maternal dose of 0.3 μg TCDD/kg bw was necessary to cause suppression in the 14 month old females. Both males and females were more sensitive to the suppression at 14 months of age than at 4 months of age. Using a higher dose of TCDD (3 μg/kg bw on GD14) it was shown that the DTH suppression persisted into old age (19 months) and that the male rats also responded with DTH suppression to keyhole limpet hemocyanin antigen challenge.

Conclusion on the studies by Gehrs *et al.* (1997) and Gehrs and Smialowicz (1998, 1999)

These studies demonstrate that prenatal exposure to 1 or 3 μg TCDD/kg bw on GD14 results in changes in thymic T-cell phenotypes, the thymus, and functional immunological suppression in the offspring. Males were more sensitive to delayed type hypersensitivity (DTH) suppression than females and the effect persisted into old age. As low as 0.1 μg TCDD/kg bw on GD14 produced slight but significant DTH suppression in male offspring 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.

Comments

The authors conclude that humoral immunity is less sensitive than cell mediated immunity as, while TCDD caused a significant suppression of the DTH response at 1 μg TCDD/kg bw, at 3 μg TCDD/kg bw there was only a non-significant suppression of the PFC assay. In this study, TCDD produced a number of thymic alterations, including altered $CD3^+$ subsets (which presumably reflects impaired T-cell maturation in the thymus and which may account for the suppressed DTH response) and a trend towards thymic atrophy and reduced thymic cellularity. Interestingly, there were splenic phenotypic changes at 3 μg TCDD/kg bw, though not at 1 μg TCDD/kg bw (thymic phenotype was not assessed at the higher dose). The authors speculate that this splenocyte alteration may be associated with suppression of the DTH response, though they do acknowledge that this suppression may be due to changes in other cell populations not examined (including thymic phenotypes). Furthermore, in study 2 there was suppression of the DTH response in the absence of any splenocyte alterations. (The $CD4^-$ $CD8^-$ and $CD4^+CD8^+$ are immature T-cells, undergoing maturation in the thymus. Alterations in the percentage of these two subsets would suggest that T-cell maturation is being impaired and therefore there is likely to be impaired cell mediated immunity, e.g. suppression of the DTH response. However, it is not clear why such subsets would be detectable in the spleen as this is not a site of T-cell maturation). While not mentioned in the results section of the paper, the authors state that severity of immune effects depends on route of exposure, i.e. group 4 > 3 > 2. However, the authors note that in humans immunological development occurs earlier than in rats so this observation may not be relevant to human risk assessment.

5.4.1. Conclusion

Prenatal exposure of pregnant rats to 0.1 μg TCDD/kg bw on GD14 represents a sensitive LOAEL for persistent suppression of delayed type hypersensitivity in the male offspring. Assuming 60% absorption (Hurst *et al*., 2000) this LOAEL would correspond to a body burden of 60 ng TCDD/kg bw.

6. Potential influence of the half-lives of PCDDs and PCDFs on WHO-TEQs

WHO (WHO, 1998; WHO, 2000) express the TDI in terms of WHO-TEQs (van den Berg *et al.*, 1998). The Committee agrees that while a number of uncertainties exist in the use of the TEF concept for human risk assessment, pragmatically it remains the most feasible approach. Use of TCDD alone as the only measure of exposure to dioxin-like PCDDs, PCDFs and PCBs would severely underestimate the risk to humans from exposure to these compounds. However, the Committee had concern that the derivation of the TEFs for individual congeners had not adequately taken into account the differences in their half lives.

One critical aspect of the use of TEFs is the possibility that the half-life of an individual congener in the human body may be sufficiently longer than that of TCDD itself to result in a greater body burden of that congener than would be expected on the basis of the half-life of TCDD. If the TEF for this congener was similar to that of TCDD and the difference in half-life was not accounted for by the TEF the effective toxicity of the congener could be greater than that predicted by the calculated TEQ.

The converse situation, in which the half-life of a congener is shorter than that of TCDD itself, would result in the effective toxicity of that congener in a mixture being less than its calculated TEQ. Therefore, in this instance the use of the assigned TEF would provide a greater margin of safety and would be the more conservative option in human risk assessment.

The Committee therefore examined available studies on half-lives of dioxins and dioxin-like PCBs in humans in order to identify congeners with half-lives greater than TCDD and subsequently consulted the WHO TEF derivation to examine whether this aspect had been covered.

The majority of the studies with dioxin congeners that have been used to determine the TEFs can be classified as sub-chronic. In addition, many of the studies were carried out with dosing schedules that did not involve daily dosing and therefore do not offer a true parallel to human exposure to the congeners, i.e. through ingestion of fats of animal origin. Kimbrough has pointed out that assignment of TEFs to the various congeners has not taken into account their half-lives, distribution and persistence. On the basis of comparisons between various incidents in which humans were exposed to mixtures in which different PCDDs, PCDFs or PCBs predominated she has suggested that the TEF values for PCDD and PCDF congeners are poor predictors of human risk (Kimbrough, 1997/1998).

A limited number of studies of the half-lives of different PCDD, PCDF or PCB congeners have been undertaken in humans. They have relied on the existence of a group of individuals who have been exposed to mixtures of congeners and have involved the repeated measurement of concentrations of the separate congeners in blood or adipose tissue samples. These are summarised below.

6.1. PCDDs and PCDFs

Flesch-Janys *et al.* (1996) examined the elimination of PCDDs and PCDFs in occupationally exposed persons. The study group consisted of a subgroup of a cohort of workers from a herbicide-producing plant in Germany with high exposures to PCDD and PCDFs. The

elimination of 2,3,7,8-substituted congeners was investigated in a group comprised of 43 exposed workers with two blood samples and 5 with three samples (early samples had been retained frozen and all were analysed at the same time). The group comprised 45 males and 3 females. The general health of the study group as well as the smoking status is documented in the paper. The measured concentrations of a specific PCDD or PCDF congener at each time point had to exceed 95% of the German background concentration of that congener for the individual's results for this congener to be included in the analysis. The mean German background concentration for each congener was subtracted from the measured concentration prior to analysis.

Graphical inspection of the data for those 5 workers with 3 blood samples showed that the loss of TCDD and 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,6,7,8-HxCDD) from the blood did not deviate significantly from first order kinetics. It is stated that this held for other PCDDs and PCDFs, but the data were not shown in the paper. The half-lives estimated from the data are shown in Table 3.

The influence of various factors, e.g. age at first sample, percentage body fat, smoking status and evidence of liver disease, on the loss of individual isomers from the body were investigated in a model of univariate regression with covariates. There was considerable variation between the different congeners in the influence of the covariate factors but, in general, increasing age and increasing percentage of body fat were associated with reduced clearance of the PCDDs and PCDFs and the clearance of the congeners by smokers was increased.

Table 3. Toxic equivalency factors and half-lives in humans of PCDD and PCDF congeners

Congener	**TEF**	**Half-life in years**		
		Flesch-Janys *et al.*, 1996	**Rohde *et al.*, 1999***	**Górski *et al.*, 1984**
2,3,7,8-TCDD	1	7.2	8.5 (22)	-
1,2,3,7,8-PnCDD	1	15.7	13.7 (27)	-
1,2,3,4,7,8-HxCDD	0.1	8.4	14.2 (21)	-
1,2,3,6,7,8-HxCDD	0.1	13.1	8.5 (22)	3.5
1,2,3,7,8,9-HxCDD	0.1	4.9	7.1 (11)	-
1,2,3,4,6,7,8-HpCDD	0.01	3.7	4.3 (15)	3.2
OCDD	0.0001	6.7	8.3 (10)	5.7
2,3,7,8-TCDF	0.1	ND	ND	-
1,2,3,7,8-PnCDF	0.05	ND	ND	-
2,3,4,7,8-PnCDF	0.5	19.6	13.7 (33)	-
1,2,3,4,7,8-HxCDF	0.1	6.2	8.25 (20)	-
1,2,3,6,7,8-HxCDF	0.1	6.0	7.6 (22)	-
1,2,3,7,8,9-HxCDF	0.1	ND	ND	-
2,3,4,6,7,8-HxCDF	0.1	5.8	10.9 (25)	-
1,2,3,4,6,7,8-HpCDF	0.01	3.0	3.7 (26)	-
1,2,3,4,7,8,9-HpCDF	0.01	3.2	ND	-
OCDF	0.0001	ND	ND	1.8

ND: No data meeting the inclusion criteria.

* Estimated from graphical results in the paper, values in parentheses are the half-lives for faecal elimination.

Rohde *et al.* (1999) conducted a mass balance study of the absorption and excretion of PCDD and PCDF congeners in six men (aged 41 to 73 years, body weights at date of study 79 to 98 kg) from the same cohort studied by Flesch-Janys *et al.* (1996). Daily, over a period of 12 days, duplicate diets of each subject and, with a lag of one day to account for passage of food through the intestinal tract, the subject's faeces were collected. The samples were pooled to provide samples of an individual's diet or faeces for four consecutive three-day periods. Two 40 ml blood samples were collected for analysis and the results compared with those of a sample taken in 1990/1992.

The concentrations of PCDDs and PCDFs in the diet of the subjects were, with one exception, comparable to those reported elsewhere for Germany. The exception was one individual who was found to be consuming contaminated eggs from home-farmed free range chickens. Unsurprisingly, the faecal samples contained generally greater concentrations of PCDDs and PCDFs than had been analysed in an unexposed German population. This effect was more pronounced for the PCDD than the PCDF isomers. Similarly, the concentrations of PCDDs in the blood were elevated to a greater extent than those of the PCDFs. The isomer pattern in the blood samples was distinct from that of the general German population.

The excretion of the individual PCDD and PCDF isomers was only considered to be significant if, during a 3-day period, it exceeded the dietary intake by a 4-fold factor. The clearance was then defined as excretion minus intake. A good correlation between faecal and blood concentrations was found and allowed the use of a first order kinetic model to describe clearance. The authors suggest that faecal excretion is regulated by lipid-based blood concentrations.

The mean half-lives for faecal elimination of individual PCDD and PCDF isomers were calculated on the basis of the data from the 3-day periods of the study. Also, using blood concentrations of isomers measured in 1990/1992 and 1996 and the calculated mass of body fat in 1990/1992 and 1996, the overall clearance rate of each isomer in the intervening period was also determined. The results are presented in Table 3; although the authors do not present individual half-lives for all the isomers in their paper some have been estimated from the graphical presentations given there.

On the basis of measurements of PCDDs and PCDFs in the adipose tissue of one chronically ill child, Górski *et al.* (1984) estimated some half-lives for different isomers. These are listed in Table 3 but it should be noted that the analytical procedure used packed column gas chromatography with electron capture detection and has not been validated.

6.2. TCDD

Five men in a cohort (157 males, 2 females) of individuals who had been occupationally exposed to TCDD during herbicide production had blood samples collected for analysis of TCDD both in 1990 and 1996. Only one of these men had a decrease of plasma concentration of TCDD consistent with a half-life of 7 years, two had only a small decrease and the concentrations in two men increased (Neuberger *et al.*, 1998).

Several studies have provided a half-life for TCDD only (Pirkle *et al.*, 1989; Wolffe *et al.*, 1994; Michalek & Tripathi, 1999). The measurements were carried out in US Air Force veterans of Operation Ranch Hand in Vietnam. In the first study serum specimens were taken from 36 veterans between 1982 and 1987, a median half-life of 7.1 years was estimated for TCDD (95% confidence interval about the median of 5.8-9.6). The criterion for inclusion in

the analysis was that neither sample from an individual should have a concentration <10 ppt of TCDD. The second study included 337 veterans of Operation Ranch Hand and yielded a median half-life for TCDD of 11.3 years with a nonparametric 95% confidence interval of 10.0-14.1 years. The third study reports calculations of the half-life of TCDD from multiple measurements of serum of 97 veterans of Operation Ranch Hand collected over 15 years. The half-life estimate was 7.6 years with a 95% confidence interval 7.0 to 8.2 years. The elimination rate decreased significantly with increasing body fat, but not with age or relative changes in percent body fat.

One human volunteer ingested a single dose of 1.14 ng/kg of tritium-labelled TCDD, 87% of the dose was absorbed from the intestine. Adipose tissue levels after 13 and 69 days were measured. The half-life of elimination of TCDD in the faeces was 2120 days (Poiger & Schlatter, 1986).

6.3. Octachlorodibenzo-*p*-dioxin (OCDD)

Two Spanish families that had been poisoned by PCDDs and PCDFs contaminating their cooking oil provided samples of blood for analysis in 1990 and 1996. There was considerable variation in the changes of concentration of octachlorodibenzo-*p*-dioxin (OCDD) in the plasma lipids of individual family members, with the parents all having higher concentrations in 1996 and there being increased, unchanged or, in most individuals, decreased concentrations in the children (Rappe *et al.*, 1998).

6.4. Dioxin-like PCBs

The Committee did not locate reliable information on the half-lives of non-*ortho* and mono-*ortho* PCBs in humans. In experimental animals (mainly rats) the half-lives of these compounds seems to be similar to or lower than that of TCDD (van den Berg *et al.*, 1994).

6.5. Conclusion

Few studies have attempted to determine the half-life in humans of a wide range of the 2,3,7,8-substituted PCDDs or PCDFs. The two that provide the best information are those of Flesch-Janys *et al.* (1996) and of Rohde *et al.* (1999). Both of these utilised the same cohort of workers that had been exposed occupationally to high levels of these compounds. The designs of the studies were different and each has its advantages and disadvantages.

For the Flesch-Janys study the advantages are that it utilised an extensive sub-group of the cohort and that all the blood samples were analysed at the same time. A disadvantage is that, with the elapse of time, the concentrations of many of the isomers that may have been present initially in the blood of the subjects will have decreased and therefore, particularly for the PCDF isomers, the numbers of samples that met the inclusion criteria for a specific congener were reduced.

The study of Rohde and colleagues has the advantage of being a mass balance study and it provides information specifically about faecal excretion, a major route of removal of the PCDDs and PCDFs from the body. It has the same disadvantage as the Flesch-Janys study in terms of the elapse of time since exposure. However, in addition, it was carried out with only six subjects for a period of only 12 days. Further, the blood analyses that were used to provide information on the overall clearance of the congeners from the individuals were carried out in separate laboratories on different occasions.

Neither study provides information on the potential variability of clearance of the PCDD and PCDF isomers from the bodies of members of the general population. The specific studies of clearance of TCDD from the body have demonstrated the variability in clearance rates that may exist. In the case of the fully chlorinated, but less toxic, congener OCDD it would appear that the variability can be extreme.

The question, as to whether there exists a PCDD or PCDF congener for which the half-life is greater than, and the TEF is sufficiently similar to, that of TCDD to raise concerns as to its influence on the TEQs calculated for population exposure, can best be answered by inspection of Table 4. It would appear that the best candidates for such congeners are 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PnCDD) with a half-life in humans of the order of 14 years and a TEF of 1 and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PnCDF) with a half-life in humans of the order of 16 years and a TEF of 0.5. These congeners, in particular 2,3,4,7,8-PnCDF, may provide an appreciable proportion of the TEQ associated with exposure to some PCDD and PCDF mixtures.

As judged from limited information 1,2,3,7,8-PnCDD and 2,3,4,7,8-PnCDF have longer half-lives (approximately 30 and 60 days, respectively) in rats than TCDD (approx. 20 days) (van den Berg *et al.*, 1994). The Committee noted that the TEF values for 1,2,3,7,8-PnCDD and 2,3,4,7,8-PnCDF have been established on the basis of liver tumour promotion studies in rats and 3-months studies of toxicity in rats and mice in which the chemicals were at or approaching steady state conditions. In these studies TCDD was included as reference compound. Thus for 1,2,3,7,8-PnCDD, WHO in 1997 (van den Berg *et al.*, 1998) recommended that the previously used TEF of 0.5 was increased to 1.0 based on new *in vivo* tumour promotion data and CYP1A1/A2 induction potencies from subchronic studies (Waern *et al.*, 1991). For 23478-PeCDF, most relative potencies (REPs) dealing with relevant toxic endpoints from subchronic studies (e.g. Waern *et al.*, 1991; De Vito *et al.*, 1997; van Birgelen *et al.*, 1996) supported the continued use of a value of 0.5.

Thus, the Committee recommends the WHO TEF approach be used for expressing the intake in humans of PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in units of TCDD equivalents (TEQs) for comparison to the tolerable daily intake (TDI) of TCDD. This will result in an approximate 10% increase in TEQ calculations, compared to using I-TEFs and the initial 1994 WHO TEFs for PCBs.

7. Should hexachlorobenzene (HCB) be included in the TEF scheme?

The Committee noted that van Birgelen (1998) has argued that hexachlorobenzene (HCB) should be classified as a dioxin-like compound, with a TEF value of 0.0001. By doing this, HCB could add 10-60% to the total TEQ in human milk samples in most countries. The Committee noted that Vos (2000) had discussed this.

To include a compound in the TEF concept, the following criteria are used: i) a compound must show a structural relationship to the PCDDs or PCDFs; ii) a compound must bind to the Ah receptor; iii) a compound must elicit Ah receptor-mediated biochemical and toxic responses; and iv) a compound must be persistent and accumulate in the food chain (van den

Berg *et al.*, 1998), criteria that are also referred to by van Birgelen (1998). It also follows from the TEF concept, that the combined toxic effects should show additivity.

The Committee questions the validity of using a TEF approach for HCB. While HCB is a persistent chemical that accumulates in the food chain and also is able to weakly bind the Ah receptor, its structural resemblance with PCDDs and PCDFs is at best weak. However, with respect to the toxic responses of HCB and dioxins it is questionable that the combined effects are dose or concentration additive. Dioxin-like effects for HCB mentioned by van Birgelen (1998) are reduction in reproduction, splenomegaly, increase in mortality, neurologic alterations, teratologic effects and immunotoxic effects.

Although TCDD and HCB share target organs of toxicity, the effects produced in these systems or organs do differ:

- Laboratory animals that are lethally exposed to TCDD die following a wasting disease. This is not seen in HCB poisoning.

- The neurotoxic effects typical for HCB in rodents and birds are tremors (Michielsen *et al.*, 1999), not observed in TCDD poisoning (McConnell, 1980).

- Teratogenic effects induced in rodents by TCDD (McConnell, 1980), but not by HCB, are cleft palate and hydronephrosis.

- Thymic atrophy and suppression of thymus-dependent immunity is a hallmark of TCDD toxicity observed in all species investigated (Vos *et al.*, 1997/1998), whereas the immunotoxicity of HCB is species-dependent, characterised in the rat and in humans by splenomegaly, enlarged lymph nodes and enhancement of parameters of specific immunity, and in the mouse by suppression of most immune responses (Michielsen *et al.*, 1999).

- Target organs differ for the carcinogenic action of HCB and TCDD, only the liver is shared by both compounds (McConnell, 1980; IPCS, 1997).

- Edema formation (not mentioned in van Birgelen, 1998) is a pathology typically caused in some species by TCDD. Notably subcutaneous edema, ascites and hydropericardium in the chicken have been reported (McConnell, 1980).

- Chloracne is a skin disease that is highly diagnostic for exposure of man to TCDD-like compounds, while cutaneous lesions in HCB poisoning are hirsutism and bullous lesions, mainly in sun-exposed skin areas. These skin lesions in victims of HCB poisoning are attributed to the porphyrogenic activity of HCB (Michielsen *et al.*, 1999).

Furthermore, for the above mentioned toxic effects of HCB, which differ largely from those induced by TCDD although in the same target organs, no literature data are available that they are Ah receptor mediated. These considerations lead the Committee to conclude that the TEF approach as defined by van den Berg *et al.* (1998) is not justified for HCB.

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