

## COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

### STATEMENT ON THE TOLERABLE DAILY INTAKE FOR DIOXINS AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS

#### Introduction

##### *Dioxins and polychlorinated biphenyls (PCBs)*

1. Dioxins are persistent organochlorine compounds that are widely dispersed environmental contaminants and which accumulate in fatty foods. The term “dioxins” is commonly used to refer to a group of 75 polychlorinated dibenzo-*p*-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, of which less than 20 are considered to be biologically active. Dioxins are produced in a number of thermal reactions, including incineration of municipal waste, domestic fires and bonfires, forest fires and internal combustion in automobile engines. They are also generated as trace contaminants during the synthesis of many organochlorine compounds (e.g. chlorophenoxy herbicides such as hexachlorophene, chlorodiphenyl ether herbicides) and during some industrial processes (e.g. bleaching of pulp and paper with chlorine gas).

2. Polychlorinated biphenyls (PCBs) are environmentally stable, lipophilic chemicals that were widely manufactured for a range of industrial applications between the 1930s and 1970s. Use of PCBs for industrial purposes has been discontinued but these substances may still be released to the environment during disposal of materials and obsolete equipment. There are 209 theoretically possible PCB congeners, of which 12 non-*ortho* or mono-*ortho* compounds exhibit similar biological activity to PCDDs and PCDFs, and are therefore referred to as “dioxin-like PCBs”.

3. There is continuing public concern about the health hazards of dioxins and related compounds. These compounds are persistent in the environment and tend to accumulate in biological systems. One of the most extensively studied PCDD congeners, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses.

4. Exposure of the general population to dioxins and dioxin-like PCBs is primarily from food. The estimated exposures from the UK Total Diet Study samples for all age groups have declined substantially. In 1982, average intakes for dioxins and dioxin-like PCBs were 7.2 and 18 pg TEQ/kg bw/day for adults and toddlers, respectively. In 1997 the averages had fallen to 1.8 and 4.6 pg TEQ/kg bw/day for adults and toddlers, respectively. <sup>1</sup> Over the same period, the intake estimates for

high level (97.5<sup>th</sup> percentile) consumers have fallen from 13 and 28 pg TEQ/kg bw/day to 3.1 and 7.2 TEQ/kg bw/day, for adults and toddlers, respectively. Dioxins and PCBs are detectable in almost all types of food. Highest concentrations are found in meat, fish, eggs and dairy products. However, cereals, fats and oils contribute significant proportions of the total dioxin and PCB intake because they are major components of the diet. The decline has been attributed in part to controls on emissions to the environment and the discontinuation of production and use of dioxin-like PCBs. It is anticipated that exposures will continue to decline with present and planned environmental controls.

5. The highest dioxin exposures in humans have generally been associated with occupational exposure or accidental contamination of the environment or edible oils. Occupational exposure studies have been undertaken at plants in the USA, Germany, the Netherlands and the UK that manufacture chlorophenols and/or chlorophenoxy herbicides. Application of chlorophenoxy herbicides has been associated with much lower levels of exposure. Exposures to more highly chlorinated PCDDs have been estimated for workers exposed to pentachlorophenol and/or other chlorophenates at saw mills or manufacturing plants. In addition, an explosion in a chemical plant at Seveso in 1976, resulted in widespread release of TCDD to the environment and exposure of the local population. The ingestion of edible oils contaminated with high levels of polychlorinated compounds including PCBs and PCDFs was associated with toxicity in food poisoning incidents in Yusho and Yu-Cheng, which we reviewed in 1997.<sup>2</sup>

### ***Previous COT evaluations***

6. The COT and our sister Committees on Carcinogenicity (COC) and Mutagenicity (COM) have considered dioxins and dioxin-like PCBs on several occasions in the past.<sup>1-8</sup> In 1989 we made a comprehensive statement about the health hazards of PCDDs and PCDFs.<sup>3</sup> We made a second statement in 1991 when UK exposure data on these compounds from food became available.<sup>4</sup> On that occasion, we endorsed the Tolerable Daily Intake (TDI) of 10 pg/kg bw/day 2,3,7,8-TCDD recommended by an expert group convened by the WHO Regional Office for Europe<sup>9</sup> and we recommended that, when considering mixtures of PCDDs and PCDFs, the TDI can be regarded as 10 pg/kg bw/day 2,3,7,8-TCDD Equivalents (TEQ). We further stated that, in view of the estimated long elimination half-lives of this class of compounds, it would be more appropriate to regard the TDI as a time-weighted average tolerable intake. We reviewed PCDD and PCDFs again in 1995, when we concluded that the new information available at that time did not necessitate the alteration of the previously agreed TDI.<sup>6</sup>

7. The Toxic Equivalency Factor (TEF) approach was initially used to facilitate risk assessment of PCDDs and PCDFs (i.e. dioxins). In 1997, we tentatively accepted that the TEF approach could be extended to include the dioxin-like PCB congeners<sup>2</sup> and in 1998 we endorsed the revised WHO-TEFs for dioxins and dioxin-like PCBs.<sup>7</sup>

## **Recent International evaluations**

### ***World Health Organisation.***

8. In 1998 the WHO European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) conducted a re-evaluation of the TDI for dioxins and dioxin-like PCBs. The Executive Summary of this report was published in a special issue of Food Additives and Contaminants<sup>10</sup>, devoted to the 1998 WHO-ECEH/IPCS Consultation on Dioxins, allowing an evaluation of the basis on which the WHO consultation reached its conclusions.

9. The WHO consultation recommended a TDI for dioxins and dioxin-like PCBs of 1-4 pg WHO-TEQ/kg based on the NOAEL/LOAELs of those effects considered to be the most sensitive in experimental animals, namely endometriosis, developmental neurobehavioural effects, developmental reproductive effects and immunotoxicity.

### ***Scientific Committee on Food***

10. The Scientific Committee on Food (SCF) undertook a reassessment of the TDI for dioxins and dioxin-like PCBs for the European Union, adopting a temporary opinion in November 2000. This was revised in June 2001, in the light of newly published data allowing calculation of the total amount of dioxin in the fetus (the fetal body burden) associated with maternal exposure at steady state.<sup>11</sup> The SCF concluded that, because TCDD and related compounds have very long half-lives in the human body, the tolerable intake should be expressed on a weekly rather than a daily basis. Based on the LOAEL from a study showing developmental effects in male rat offspring following repeated subcutaneous administration of TCDD<sup>12</sup>, the SCF established a tolerable weekly intake (TWI) of 14 pg WHO-TEQ/kg bw.<sup>13</sup>

### ***Joint FAO/WHO Expert Committee on Food Additives***

11. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) also considered dioxins and dioxin-like PCBs in June 2001. The JECFA used a similar body burden approach to that used by the SCF and also took into account exposure from background contamination considered to be present in feed provided to laboratory animals. It proposed a provisional tolerable monthly intake (PTMI) of 70 pg WHO-TEQ/kg bw<sup>14</sup>, based upon the lowest LOAEL and a NOAEL for developmental effects in male rat offspring.<sup>12, 15</sup>

### ***Environmental Protection Agency***

12. The U.S. Environmental Protection Agency (EPA) commenced a reassessment of dioxin exposure and human health effects entitled, "Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds" in April 1991. In 1994, it released its initial external review draft describing health effects and exposures. In our 1995 statement<sup>6</sup>, we welcomed the US EPA initiative to investigate further the health hazards of 2,3,7,8-TCDD and related compounds. The document provided a thorough review of the literature on the effects of these compounds on various biological systems. However, we did not

consider it provided any new information or analysis that necessitated the alteration of the previously agreed TDI of 10pg TEQ/kg bw/day or of our previous advice.

13. Following public comment and advice from its Science Advisory Board, the EPA undertook an extensive revision of its review, particularly two key sections on Dose-Response Modelling and Integrated Summary and Risk Characterization.<sup>16</sup> These chapters were subject to public comment and further Science Advisory Board review during late 2000 and Spring 2001. The EPA's draft assessment considered that cancer was the most appropriate end-point for risk assessment and undertook an evaluation based on their risk assessment guidelines for carcinogens. The results of this evaluation are not yet available.

#### **Evidence considered in the current evaluation.**

14. In 2000, we were asked to review the risk assessments of dioxins carried out by the WHO, the SCF, and the US-EPA. We concluded that it was appropriate to conduct our own evaluation of the data, informed by these international assessments and other relevant information, before reconsidering the TDI for dioxins and dioxin-like PCBs. As part of this evaluation it was essential that the evidence concerning human cancer risks for dioxins be evaluated to determine whether or not it is appropriate to assume the existence of a threshold and hence whether a TDI could be established. We are grateful for the assistance provided by the COC and we have taken account of its conclusions<sup>17</sup> in completing our evaluation.

15. In undertaking our evaluation we have had access to the published assessments of all three international evaluations. The EPA documents provided the most detailed and comprehensive review of the published literature. We have supplemented this by evaluation of the original publications identifying critical end-points and recently published data.

#### **Mechanism(s) of action of TCDD**

16. Most of the actions of TCDD and related compounds can be ascribed to the consequences of an initial binding to what has become known as the Ah or aryl hydrocarbon receptor (AHR), although this binding protein is now more properly termed a ligand activated transcription factor. This binding results in multiple changes in gene transcription leading to increases in biotransformation enzymes, modulation of cell cycling proteins and other responses. Inappropriate gene expression resulting from the high affinity binding and long term occupancy of the receptor may be the basis of the toxicity of TCDD. However, although the mechanisms of early molecular changes are well understood, the relationship between changes in gene regulation and observed toxicity is still unresolved.

17. It has become apparent that the sequence of events from TCDD binding to gene transcription involves other transcription factors, chaperones (such as HSP90) and regulatory proteins. The net result is the association of TCDD-AHR with another factor, the Ah receptor nuclear translocator (ARNT), in the nucleus followed by binding of the complex to 'dioxin' responsive regulatory elements (DREs) in enhancer regions upstream of particular genes. Downstream activation of promoter

regions then occurs with production of mRNA from the genes. Most of the molecular events for transcription of the CYP1A1 gene have been elucidated. For other genes the sequence of events is far less clear but probably occurs in a similar manner<sup>18</sup> and the number of known AHR-regulated genes is still increasing.

18. Mechanistic studies on the role of the AHR in the toxicity of TCDD have shown that proteins similar to the AHR have been found in many organisms suggesting that this receptor has an essential biological function<sup>19</sup>. Sequencing studies on the AHR have shown that it is a member of a family of gene regulating proteins known as PAS (PER-ARNT-SIM)<sup>20</sup>. In mammals, these proteins (which include hypoxia inducible factor 1- $\alpha$  [HIF1 $\alpha$ ] and ARNT) regulate the transcription of specific genes. Heterodimerisation of the AHR with ARNT is apparently essential for the TCDD activated AHR to induce specific DNA binding and transactivation *in vitro*. However, heterodimerisation of ARNT can also occur with HIF1 $\alpha$  and with a newly discovered factor AHR repressor (AHRR).<sup>21</sup> TCDD/AHR might act by competing against these, or even competing against the binding of a hypothetical normal endogenous ligand of the AHR that has yet to be found. Other studies have shown that levels of the AHR, AHRR, ARNT and HIF1 $\alpha$  may be regulated by cell type and activation and by stages of growth and differentiation.<sup>22</sup>

19. A number of lines of evidence *in vivo* support the role of AHR in TCDD toxicity. For instance, polymorphism of the AHR, with varying affinities for TCDD, in mice correlates with variable susceptibility to toxic effects.<sup>23</sup> Different strains of mice that do not possess the functioning gene for the AHR (referred to as *Ahr* null or 'AHR knockout' mice) have been shown to be extremely resistant to very high doses of TCDD for a variety of toxic endpoints. Binding capabilities of dioxins and dioxin-like PCBs to the AHR, as shown by structure activity relationships, generally show similar ranked order to their elicitation of biochemical responses.

20. However, in seeking to understand the mechanisms of action in order to inform risk assessment, it might be inappropriate to place exclusive emphasis on the AHR. At very high doses of TCDD (in the *Ahr* null mouse) the chemical may have toxic actions which are not mediated by the receptor. Similarly, in some *in vitro* experiments, various effects of TCDD have been interpreted as non-AHR dependent.

21. In terms of binding to the AHR, some ligands may be competitors of TCDD-induced gene regulation. Conditional disruption of the *Arnt* gene has recently shown that ARNT is required for AHR-stimulated gene activation by TCDD in liver, but this association has yet to be extended to toxicity.<sup>24</sup> Other, as yet unidentified, AHR ligands may be present, or TCDD-AHR complex may participate in cell dysfunction by unknown routes not involving the regulation of gene expression via DREs and ARNT. Although no endogenous ligand of AHR has been proven, a number of naturally derived chemicals are ligands.

22. Some data suggest that the binding affinity, and the effect of binding, of TCDD to the AHR, are much lower in humans than in rodents, even the resistant DBA/2 mouse.<sup>25</sup> This could contribute an extra safety margin but the difference in response may vary with endpoint. Some polymorphisms of the human AHR gene have been reported but the functional significance of these polymorphisms is still under

investigation.<sup>26</sup> We note that, in view of this uncertainty, it is not possible to exclude the possibility that the most sensitive humans are as responsive as the most sensitive rodents. Overall, we agree that the evidence that toxicity is mediated via the AHR, and the limited evidence that dioxin/receptor interaction does not inevitably lead to a toxic response, are sufficient to consider a threshold approach to the risk assessment.

## **Toxicokinetics of dioxins**

### ***Absorption***

23. The extent of gastrointestinal absorption of dioxins is reported to vary with the medium or vehicle of administration, and the lipophilicity of the individual congeners. The percentage absorption of TCDD is approximately 60% in rodents.<sup>27</sup> Similar absorption has been reported for other chlorinated dibenzodioxins and dibenzofurans, although the absorption of octachlorodibenzodioxin (OCDD) is less than 20%. PCDDs and PCDFs are incompletely absorbed because they are not in solution within the gut lumen, and absorption is dependent on the digestion and emulsification of the food matrix.

### ***Distribution***

24. Once absorbed, probably via chylomicrons, TCDD rapidly leaves the blood compartment with a distribution half-life of approximately 30 minutes in rats, after which time it is primarily found in adipose tissue, the liver, skin, muscle and other tissues. TCDD present within the blood is largely associated with lipoproteins. Studies in rats have shown that after initial rapid distribution there is a slower redistribution from muscle and other organs, primarily to the liver and adipose tissue, skin and thyroid gland; the concentrations in these organs show a slow increase over a period of about 4 days following a single intraperitoneal dose.<sup>28</sup> This pattern of distribution is probably representative of distribution in humans and there is a high correlation between adipose tissue concentrations and the levels in serum.

25. The duration of the distribution phase is very short compared with the elimination phase. After tissue distribution, which takes about 4 days, *in vivo* elimination is adequately represented by a single mono-exponential decrease and half-life. The distribution phase is important in the interpretation of effects produced *in utero* in rats after a single oral dose given late in pregnancy, which are the basis for determining the tolerable intake.

### ***Metabolism and elimination***

26. Although early studies suggested that TCDD is not metabolised, it is now recognised that it is slowly converted to polar metabolites that are eliminated as glucuronides. The main metabolites of TCDD formed with rat hepatocytes *in vitro* are 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TCDD. Other metabolites have been identified in dog, including tri- and dichloro-hydroxy and dihydroxy- compounds. Oxidative metabolism does not appear to give rise to significant bioactivation or formation of DNA adducts and the limited available data indicate that the metabolites are less toxic than the parent compound.<sup>29</sup> A major route of elimination of the hydroxy- metabolites is as glucuronic acid conjugates in the bile. Unmetabolised

PCDDs and PCDFs are not detected in bile but are excreted in the faeces and faecal fat by direct intestinal elimination.

27. The half-life of TCDD has been reported to be about 20 days in the rat, 12 days in mice, 90 days in the guinea pig and between 6 and 11 years in humans.<sup>28,30-32</sup> The elimination half-life in humans correlates positively with the percentage body fat, indicating slower elimination in individuals with higher fat composition. Consistent with this is evidence suggesting an age-related decrease in half-life in the elderly, as the fat stores are mobilised during redistribution from the subcutaneous to abdominal areas<sup>33</sup>. Mobilisation of fat stores during lactation contributes to the presence of dioxins in breast milk<sup>34</sup>, and this is associated with a decrease in the maternal body burden during breast-feeding.

## **Human data**

### ***Introduction***

28. The human effects observed in one or more studies are summarised in Table 1. In assessing the effects of dioxins and dioxin-like PCBs in humans, we have selected those studies that provide the most information on the relationship between outcomes and exposure to TCDD-contaminated materials. Case reports were not reviewed and only cohort studies with calculated standardised mortality ratios (SMRs) or equivalent, were discussed in assessing mortality. Many of the studies reviewed are cross-sectional in design and there are inherent limitations of this type of study. We noted that the lack of adequate exposure data was a frequent limitation of the available epidemiology. Exposure is measured in different media and expressed in different units across the studies, which makes comparison difficult. Some studies were only able to use indirect estimates of exposure, which cannot be directly related to dioxin levels. Development of a tolerable intake requires studies with quantitative assessment of exposure.

29. We focused our evaluation on studies in which exposure was assessed by measurement of dioxin concentrations in serum or body fat, which could be correlated with body burden and intake. The body burdens in human studies have been estimated in two ways. Firstly, the body burden may be calculated from the concentrations of dioxins in lipid and the percentage body composition as fat. This does not allow adequately for sequestration of dioxins within the liver, but this should produce only a minor error in the calculation of body burden. The second method is calculation of the body burden based on estimates of intake and half-life. In humans the intakes of dioxins will have varied historically and there is uncertainty about past exposures. In addition, little is known about the half-life of dioxins and dioxin-like PCBs at different life stages. Calculation of body burden based on daily intake has to allow for the bioavailability from the food matrix and the half-life or clearance from the body. A limitation to this method for considering mixtures of dioxins and dioxin-like PCBs is that reliable estimates of the half-life of TCDD and also its congeners are necessary.

30. We also focused on the relationship between exposure and response, particularly (although not exclusively, if other important health end points were

investigated) for the health end points that are relevant/comparable to the results of animal studies. We noted that the EPA report identified six studies or series of studies in humans, which measured serum levels of TCDD and compared them with possible health effects.<sup>35-43</sup> We were also informed of an additional study in Dutch chemical workers, which provided exposure data<sup>44</sup> and a series of Dutch studies on cognitive development.<sup>45-53</sup>

*Table 1. Effects associated with human exposure to dioxins.*

<b>Effect</b>	<b>Epidemiological evidence</b>
Chloracne	Proven association No clear dose relationship
Gastrointestinal effects and liver enzymes	Transient increases in some liver enzymes
Cardiovascular diseases	Positive association in occupational studies, but not in airforce veterans exposed to herbicides in Vietnam (Operation Ranch Hand). Dose-response in some studies
Changes in lipid levels	Results not consistent
Diabetes	Overall results not consistent Increased risks of morbidity in Seveso and Ranch Hand study
Reproductive hormones	Inconsistent results
Reproductive outcomes	Change in sex ratio of offspring with highly exposed fathers in Seveso No data yet on possible effects such as endometriosis and fertility in women – Seveso endometriosis study on-going
Thyroid function	Results not entirely consistent. Some small differences reported in thyroid hormone uptake levels.
Neurological / psychological effects	Inconsistent findings. Some effects reported in Ranch Hand study and Seveso (polyneuropathies, abnormal co-ordination) No association with depression
Respiratory system	Inconsistent evidence Irritative effects and reduced lung function in some studies
Urinary system	No major renal or bladder dysfunctions observed.
Immunological effects	Inconsistent findings.
Neurobehavioural developmental effects	Some observed differences in on-going Dutch studies
Cancer	Regarded as a probable human carcinogen (based on human, animal and mechanistic data)

31. With the exception of the series of Dutch studies on cognitive development, the studies reported the effects of high level occupational exposure or the results of accidental release. Occupational or accidental exposure would be associated with higher peak body burdens, followed by gradual elimination and were therefore

difficult to compare with steady state conditions associated with background human exposure via the diet or with repeated exposure in animal studies. Also, the occupational studies have not addressed the reproductive effects that represent the most sensitive endpoints in the animal studies.

### ***Studies of cognitive development***

32. A series of Dutch studies involved cohorts in Rotterdam and Groningen, representing a highly industrialised region and a less industrialised, more rural area, respectively. The cohorts were sub-divided between breast-feeding for a minimum of six weeks and formula fed using a single batch of one commercial formula. Plasma from maternal and cord blood samples and milk samples were analysed for dioxins and PCBs, including some PCB congeners considered not to have dioxin-like properties. The infants were monitored at ages from 3 to 42 months, with assessments of motor and cognitive development, as well as indicators of thyroid function.<sup>46-52</sup> Similar studies were conducted on a smaller cohort in Amsterdam.<sup>53</sup> We invited additional expertise to ensure that these studies were reviewed adequately, particularly the relevance of the methodology, and we gratefully acknowledge the assistance provided.

33. We noted that the measures used were standard for the age of children assessed, and the best available in the absence of an *a priori* hypothesis of specific effects. However, we were informed that in very young children it is only possible to perform crude tests which do not provide a clear distinction between motor and cognitive development. Such tests therefore serve more as screening tests than definitive measures and thus the interpretation of any observed change may be hard to assess. Tests of motor function had been conducted from shortly after birth until about 30 months of age. Tests of cognitive function were conducted from 3 months to about 42 months. Different patterns had been observed in the studies conducted at different ages and very little change was observed in the middle of the age range. We noted that Prechtl's neurological examination (as conducted on the Rotterdam and Groningen cohort<sup>47,48</sup>) was considered the most stringent, but with the disadvantage of generating a large number of false positives. For infants, the Bayley Scales, based on a very large sample and well standardised, is probably the best instrument. This scale is divided into a mental development index (MDI) that measures how motor tasks (e.g. control of hands) are applied and a psychomotor index (PSI) that measures gross movements (e.g. walking). However, the Bayley Scales provide a measure of timing of appearance of certain skills and not the quality with which they are carried out. We noted that the study used a 1969 version of the test whereas a more advanced version was published in 1993.

34. The paper of Patandin *et al.*<sup>52</sup> reported changes at 42 months using the Kaufman Assessment Battery. This was considered to be critical to our assessment, since cognitive function is stabilising at this age and becomes predictive of function in later life. In contrast, Bayley scales are used for younger age groups (3 months to 3 years) and have low predictivity. The Kaufman scores at 42 months suggested an effect of pre-natal dioxin exposure leading to an effect on cognitive development.

35. It was difficult to determine whether the effects were due to dioxin exposure or to confounding factors. Complex correlations were found between dioxin and PCB levels and confounding factors, such as breast-feeding, smoking and maternal education. Linear regression analysis had been used to assess the influence of confounding factors. It was not clear whether this was appropriate, and the data were insufficient to determine whether the statistical approach might result in over- or under-correction. Overall, if the effects were real, they were most likely to be due to pre-natal exposure. Breast-feeding ameliorated the effects. However concerns over the known and potential confounders made it impossible to reach firm conclusions.

36. We noted that distinction between pre-natal and post-natal exposure to dioxins and PCBs was an issue of concern relating to the Dutch studies. Prenatal exposure was based on analyses at 8 months gestation, and it was not clear whether these were fully representative of exposure throughout pregnancy. However, when considering effects on thyroid function it should be noted that these effects may be confounded by changes in maternal thyroid hormone production prior to thyroid development in the fetus. None of the populations examined in the Dutch studies were considered to have exposures greater than the normal background range, differences were found between industrial and rural locations and there was a very large natural variation. In addition breast-feeding appeared to be a major confounder, with the highest proportion of breast fed infants having the highest dioxin concentration. Similarly level of maternal education appeared to correlate best with high exposure as did smoking. The paucity of information on the mathematical models used in the study made it impossible to determine whether effects were "real" or due to confounders.

37. We concluded that it was not possible to determine whether any cognitive changes represented temporarily delayed milestones of development or a persistent decrement and that follow-up studies were needed. These should be carried out two to three years after the original study or during the teenage years, as increasingly sensitive measures can be used in older children. Decreased variability in older children also tends to make the tests more sensitive. In the absence of such studies we do not consider it possible to come to clearer conclusions about the outcome of dioxin exposure.

### ***Sex ratio.***

38. A recent study has reported on the sex distribution of children born between 1 April 1977 and 31 December 1996, with one or both parents exposed to TCDD in the Seveso incident, for whom TCDD serum concentrations were available relating to the time of the incident<sup>54</sup>. The exposed individuals were between 3 and 45 years old in 1976. Compared with an unexposed population, there was a dose-related decrease in the proportion of male children born to TCDD-exposed fathers. We note that this difference was statistically significant at paternal serum TCDD concentrations of 118 pg/kg or more. This could be estimated to correspond to a body burden of 24 ng/kg bw, which is equivalent to a daily intake of 12 pg/kg bw/day. However, the high exposure resulting from the Seveso incident is not comparable to steady state exposure, and the body burden derived from the peak serum concentrations in 1976 may not be the most appropriate dose surrogate for reproductive effects occurring in subsequent years.

### ***Endometriosis.***

39. Eskenazi and co-workers published initial details of the Seveso Women's Health Study<sup>55</sup>. The primary objectives of this study are to investigate whether there is a relationship between TCDD exposure and the following end-points; endometriosis, menstrual cycle characteristics, age at menarche, birth outcomes of pregnancies conceived after 1976, time to conception, clinical infertility and age at menopause. Insufficient results are currently published to assess the effects of TCDD exposure in the Seveso incident on endometriosis and other reproductive end-points in women. We considered that further consideration of female reproductive outcomes should be deferred until further papers on the Seveso Women's Health Study become available.

### ***Immunotoxicity.***

40. We noted that, compared with studies in experimental animals, there is much less information regarding immunotoxicity in humans. Nevertheless, there are suggestions that human immune function may be less susceptible to TCDD and dioxin-like PCBs than that of rodents.

41. Evidence for immunotoxicity in humans resulting from occupational or accidental exposure to TCDD or related PCBs is inconsistent. However, a common feature of some investigations has been a modest exposure-related reduction in the frequency of peripheral CD4 T lymphocytes. The extent to which these effects represent an early indication of immunosuppression is unclear.

42. A recent paper has examined infectious and atopic diseases and immunological parameters in children with background levels of exposure to PCBs.<sup>56</sup> These are the cohorts from Rotterdam discussed in paragraphs 32 to 37, above. A large number of analyses are reported, many of which are simply correlation coefficients and some of the statistically significant results are likely to have occurred by chance. The authors concluded that exposure to PCBs and dioxins might be associated with a greater susceptibility to infectious diseases and a lower prevalence of allergic diseases. However, we noted a number of contradictions in the reported results, in addition to the uncertainty over control for confounders as noted in paragraph 35. We concluded that the study did not provide convincing evidence of a causal relationship between pre-natal exposure or total body burden to PCBs and increased susceptibility to infectious diseases or decreased incidence of allergic disease.

### ***Cardiovascular disease***

43. Some studies have reported a positive association between exposure to TCDD, or to PCDDs and PCDFs, and the incidence of ischaemic heart disease.<sup>39,40,42,44</sup> These studies have indicated that a significant increase in ischaemic heart disease is associated with a body burden at or above 25 ng TCDD/kg bw, or 55 ng TEQ/kg bw for PCDDs and PCDFs. However, they did not adequately allow for confounding by other risk factors, such as smoking and diet. No studies included measurement of dioxin-like PCBs exposure or its contribution to the body burden.

## **Cancer**

44. The Committee on Mutagenicity (COM) and the Committee on Carcinogenicity (COC) considered 2,3,7,8-TCDD in 1988/9 and concluded that this compound was carcinogenic in rodents but that this was unlikely to be due to a mutagenic mechanism. The COC gave further consideration to the carcinogenicity of 2,3,7,8-TCDD in 1993, when more epidemiological data were available. The Committee concluded that the new data strengthened the possibility of an epidemiological link between occupational exposure to 2,3,7,8-TCDD and an increase in total cancers in humans, although there was no consistent association with cancer at any specific anatomical site(s). It was considered that there was insufficient evidence for a clear causal link but it would be prudent at present to regard 2,3,7,8-TCDD as a possible human carcinogen<sup>5</sup>.

45. The COC reviewed TCDD in 1998, following the publication of the IARC monograph which concluded that TCDD should be considered as a definite human carcinogen<sup>57</sup>. The COC agreed that TCDD is a potent carcinogen in laboratory animals, but that the information from the most heavily occupationally exposed cohorts suggested that there was, at most, only a weak carcinogenic effect in these individuals. It therefore concluded that there were insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but it would be prudent to consider TCDD as a “probable weak human carcinogen”.<sup>58</sup>

46. The COC has reconsidered its 1998 statement in the light of recently published data on cancer epidemiology, including the twenty-year follow-up of the Seveso incident<sup>59</sup>, and mechanisms of carcinogenicity. It agreed that TCDD should be regarded as a “probable human carcinogen” on the basis of all the available data. The COC agreed that although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (i.e. negative genotoxicity in standard assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate. The COC did not consider it possible to quantify the margin-of-safety risk assessment in view of the difficulties in selecting the appropriate metric of exposures. However, it noted that the excess cancer mortality reported in the heavily exposed industrial cohorts was small and commented that any increased risk of cancer at background levels of exposure is likely to be extremely small and not detectable by current epidemiological methods.<sup>17</sup>

## **Animal data**

47. There are few regulatory rodent toxicity studies and no regulatory non-rodent studies on the dioxins, and most of the available data relate to TCDD. Most of the regulatory toxicity studies were performed at least 20 years ago and cannot be considered adequate for the determination of NOAELs. The recent studies were conducted to non-standard protocols and many of the studies examining the most sensitive end-points also failed to identify NOAELs. We have reviewed the experimental toxicology of TCDD, with particular consideration to those showing effects at the lowest doses.

### ***Immunotoxicity.***

48. We noted that the available data presented a complicated picture, with diverse protocols, including the use of different species and strains; various routes and durations of exposure and a wide range of doses. Nevertheless, some general points could be made:

49. In rodent studies the most consistent effect is a reduction in antibody responses to sheep red blood cells (SRBC). The SRBC assay is primarily a measure of the integrity of humoral immunity. However, as initiation and maintenance of antibody responses to SRBC requires not only B lymphocytes, but also functional T lymphocytes and antigen processing/presenting cells, this assay provides something of an overall view of adaptive immunity.

50. The most sensitive adverse effect level resulting from exposure to TCDD in which an immune alteration has been implicated was reported by Burleson *et al*<sup>60</sup>. An increased mortality of mice following challenge with influenza A virus was found following a single exposure to 0.1, 0.05 or 0.01 µg/kg TCDD. However, there is no evidence that the observed increase in susceptibility to virus challenge was necessarily attributable to impaired immune function and mortality was not associated with increased titres of virus in the lungs of mice exposed to TCDD. Therefore it could not be concluded that the lowest dose in this study represents the LOAEL for TCDD-induced immunotoxicity in mice.

51. We concur with the conclusion of the WHO, EPA and SCF reviews in considering the studies of Gehrs and colleagues to be important in assessing the immune effects of dioxins.<sup>61, 62</sup> Pregnant rats (Fischer 344 strain) received a single oral dose (on gestational day 14) of 0.1, 0.3, 1.0 or 3.0 µg/kg TCDD. Exposure at all doses was associated with a persistent (up to 14 months) reduction in males of delayed-type hypersensitivity (DTH) responses to bovine serum albumin. Maternal doses of 0.3 µg/kg TCDD and above were required for persistent suppression of DTH reactions in female offspring. On the basis of these investigations it is likely that 0.1 µg/kg TCDD should be regarded as the LOAEL for immune effects in young rats.

52. A second conclusion drawn from these studies was that maximal inhibition of immune function required both lactational and *in utero* exposure. This was more effective than lactational exposure alone, which was in turn more effective than *in utero* exposure only. It was noted that these differences in potency related to rats and might differ in humans.

### ***Developmental and reproductive toxicity***

53. The studies on developmental and reproductive effects in experimental animals mainly involved administration of TCDD alone, but there were comparative data for other congeners on teratogenicity and ovarian function. TCDD was able to elicit a number of different developmental effects although the sensitivity differed. The most sensitive and robust end-point was the effect on epididymal sperm count.

54. The EPA provided an excellent comprehensive review of the literature on developmental and reproductive toxicity, and although some new studies had emerged since it was written these did not have a major impact. The human sensitivity (based on *in vitro* data on embryonic AHR concentrations in different species) appeared to be in the middle of the range shown by experimental animals. Whilst the AHR was clearly implicated in the teratogenicity of TCDD, its role in other developmental effects was less clearly established. The reproductive effects were correlated with body burden at the critical stage of sexual differentiation (GD 15-16, as noted by SCF and JECFA<sup>13,14</sup>) and it appeared that equivalent fetal body burdens on day 16 of gestation were achieved by administration of different bolus doses on day 8 and day 15 of gestation.

55. We noted that the most sensitive end-points were observed following bolus administration and paid careful consideration to the relevance in deriving a tolerable intake. These studies are considered in detail in paragraphs 64-70. We noted that there was evidence to support an extrapolation from a bolus dose to a chronic exposure, as considered in paragraphs 71-74. The only multigeneration study was old<sup>63</sup> and was subjected to detailed evaluation in previous considerations by the Committee.<sup>4</sup> We considered that the results from this multigeneration study supported the body burden estimates but that there were questions about the statistics which required further evaluation.

56. We were informed that data from animal developmental studies did not show differences in the sex ratio of offspring, as had been reported for humans in the Seveso region. However, we accepted the animal studies were not designed specifically to address this issue.

### ***Endometriosis.***

57. In our 1995 statement we noted a study reporting an increased incidence of endometriosis in rhesus monkeys 10 years after completion of a study in which TCDD was administered in the diet for a period of about 4 years.<sup>64</sup> A recently published paper follows up the same group of monkeys 13 years after completion of the dietary study, reporting that the incidence of endometriosis correlated with serum levels of certain PCB congeners, but not TCDD.<sup>65</sup> Monkeys involved in a study in which lead was administered were also found to show an association between serum PCB levels and endometriosis. The authors could not account for the source of PCB exposure to these animals.

58. We noted that a number of aspects of this observational study undermined confidence in the results and in the earlier findings and concluded that it was not possible to draw reliable conclusions.

### ***Acute, subchronic and chronic toxicity***

59. TCDD causes a wide range of toxic responses after short and long term exposure with large differences in sensitivity between species/strains of animals to particular responses. Most of the reported toxic responses could be produced in every species provided an appropriate dose was given. The wide variability in sensitivity and the particular toxic response produced within and between species,

makes it difficult to identify an appropriate endpoint for risk assessment. Lethality (as determined by LD50) varies with species from the highly sensitive guinea pig to the relatively insensitive hamster. There is also considerable variation within species. The value of these studies for risk assessment is doubtful given the age of the various studies, and the use of different strains, dosing regimens, routes of administration and observation period. No single site of toxicity has been identified as the cause of lethality; each species has a different spectrum of organ toxicity with a wasting syndrome and hepatotoxicity as the most common features. The wasting effect occurs in several species, but no single explanation for this effect has been described. Hepatotoxicity includes a wide range of liver effects in many species with rats and mice at the sensitive end and guinea pigs and hamsters as the least sensitive species. There is considerable variation in response within different strains of rat. The chronic dietary administration studies of Kociba *et al.*<sup>66</sup> reported that the lowest dose of 0.001 µg/kg bw/day was a NOAEL for hepatocellular nodules, although low body weights were recorded at various times during the study and only animals surviving to the end of the study were necropsied. In this study, the tumour incidences were significantly increased at a number of sites at the 0.1 µg/kg bw/day dose level.

60. We noted that there was no adequate basis for decisions on acceptable risk levels in humans based on the standard toxicity studies. Two of the most sensitive endpoints across the species seemed to be induction of CYP 1A1 and oxidative stress. Although CYP 1A1 induction is not considered to be a toxic response, it could underlie toxicity resulting from disruption of various endogenous processes. However, we noted that induction of CYP isozymes does not always show a good correlation with responsiveness in different mouse strains, indicating that it cannot be directly linked to toxicity. Oxidative stress had been detected in mouse brain<sup>67</sup>, although it was not clear whether this was related to CYP induction.

## **Overall assessment**

### ***Use of body burden as a dose surrogate.***

61. We considered that the most appropriate measure of exposure for assessing the sensitive endpoints of TCDD toxicity were the associated tissue concentrations, rather than the administered dose. Ideally the concentration in the target tissue would be the most appropriate measure of dose for comparing effects in different species, but this is impracticable for humans. The tissue concentration is directly related to the body burden at steady-state so that calculated body burdens are a valid surrogate. We therefore consider that the exposure/dose-response relationship for TCDD and related compounds should be based on body burden not external dose. The body burden approach allows for the massive interspecies differences in the half-life, and the potential for accumulation. An additional advantage of using body burdens, compared with previous dose-response assessments based on external dose, is that the body burdens can be estimated for occupational and accidental exposures, and body burden-response relationships assessed. We concur with the recent evaluations that, despite some limitations, the body burden provides the appropriate dose metric, and that there is sufficient scientific evidence to support the use of body burden.

## ***Human daily intakes and body burden***

62. Following dietary exposure to dioxins and dioxin-like PCBs, the body burden will be accumulated over a period of 15-30 years in humans, during which time the environmental concentrations of these substances have decreased. In consequence, the body is not truly at steady-state, and hence there will be errors in the daily intake when calculated from current concentrations in body lipids. A pharmacokinetic model that allows for decreasing environmental concentrations with time indicates that the simple steady-state assumption over-estimates daily intake by approximately 20%. Some equations relating daily intake to body burden (based on adipose levels) do not include a specific term for bioavailability, and this would need to be considered for each route/protocol for exposure. This analysis is particularly important in relation to interpretation of human epidemiology studies where the body burden and daily intake is based on analysis of adipose tissue concentrations.

63. Overall, the data indicate that dioxins and dioxin-like PCBs may be associated with a number of effects, including cancer and cardiovascular disease, but generally at body burdens at least 10-fold higher than those occurring in the general population. Most of the studies involve groups that have been exposed to very high levels of dioxins resulting from occupational or accidental exposure and the pattern of exposure does not reflect long-term dietary exposure.

## **Evaluation**

### ***Key studies***

64. We conducted a detailed review of the human data linking health effects to dioxin exposure, and a summary of these data is available on the COT website (<http://www.food.gov.uk/multimedia/pdfs/cot-diox-epi.pdf>). We concluded that the available human data did not provide a sufficiently rigorous basis for establishment of a tolerable intake. This was because:

- the epidemiological studies do not reflect the most sensitive population identified by animal studies,
- there are considerable uncertainties in the exposure assessments and inadequate allowance for confounding factors;
- the patterns of exposure did not reflect exposures experienced in the general UK population, which are mainly from diet.

We therefore found it necessary to base our evaluation on the data from studies conducted in experimental animals.

65. In accordance with the advice of the COC<sup>17</sup>, we considered it appropriate to take a threshold approach to establishing a tolerable intake. This is based upon the negative genotoxicity in standard assays and evidence from studies of mechanisms.

66. Because a threshold-based approach was considered appropriate, we examined all of the toxicological effects, in addition to cancer, in order to identify the most sensitive end-points. We concluded that the most sensitive indicators of TCDD toxicity were the effects on the developing reproductive systems of male rat fetuses

exposed *in utero*. These data were used despite inconsistencies in the findings reported, and the fact that none of the recent observations were made following sub-chronic or chronic dietary administration that would give constant (steady-state) maternal body burdens. We note that tolerable intakes were also derived from these endpoints in the recent SCF and JECFA evaluations. The key studies used different strains of rats and tended to give contradictory findings. A change in urogenital distance was found after single oral doses given on day 15 of gestation (GD15) of 50ng/kg bw<sup>15</sup>, 200ng/kg bw<sup>68</sup> and 1000ng/kg bw<sup>69</sup>. We considered that the data on ano-genital distance were not robust because of lack of correction for body weight or other means of normalisation, and should be regarded as an intermediate marker with no functional significance. Decreases in sperm numbers, production, reserve or morphology were found after single oral doses of 50ng/kg bw and above (GD15)<sup>68-70</sup> and subcutaneous dosage to give a body burden of 25ng/kg bw<sup>12</sup>, but not, in one study, at 800ng/kg bw (oral dose on GD15)<sup>15</sup>. Changes in the weight of the urogenital complex, including the ventral prostate were reported after an oral dose of 200ng/kg bw on GD15<sup>15</sup> but not at 300ng/kg bw subcutaneously<sup>12</sup>.

67. Despite some inconsistencies, we considered that the effects on sperm production and morphology represented the most sensitive effects. These were indicative of the functional adverse reproductive effects in the rat that were produced by long-term administration in the multigeneration study of Murray *et al* at doses resulting in a 10-fold higher body burden than those in the studies of sperm production<sup>63</sup>. We also note that the sperm reserve in the human male is much less than that in the rat, and therefore these changes are considered relevant. No NOAEL was available for these effects, but the study of Faqi<sup>12</sup> provided the lowest LOAEL. We noted limitations in this study but considered that the results could not be discounted and therefore, that this should be used as the basis for deriving the tolerable intake.

68. We considered that a tolerable intake based on these effects would also protect against any risk of carcinogenicity from dioxins and dioxin-like PCBs. This conclusion is based on the mode of action of dioxins and difference between the body burdens at background levels of exposure and those associated with increased cancer risk as observed by the COC<sup>17</sup>.

69. Three of the studies<sup>15,68,70</sup> reported adverse effects in male rat offspring following a single oral dose of TCDD given on GD15, and one<sup>12</sup> following repeated weekly subcutaneous injections. In all cases the effects were observed postnatally and the pattern of both *in utero* and post-natal exposure would be different. Because of the long half-life of TCDD (21 days in rats), and its presence in milk, the male offspring would be exposed to decreasing concentrations until the time of measurements. The recent SCF and JECFA evaluations<sup>13,14</sup> used recently published toxicokinetic studies<sup>11,27</sup> that allow the fetal body burdens to be calculated on GD16, on the assumption that this is the appropriate site of action, and period of sensitivity.

70. We have adopted a similar approach to the SCF and the JECFA. However, in view of the numerous assumptions in this approach (described below), we have used a simplified calculation of fetal and maternal body burdens associated with these different dosage regimens and their conversion to the steady-state dietary

intakes that would result in the same fetal body burdens. Calculation of a tolerable intake for humans is complex and requires a number of steps: calculation of the fetal body burden of rats under the experimental conditions; correction of the corresponding maternal body burden in rats to represent chronic daily intake *via* the diet; the use of uncertainty factors to give an equivalent tolerable human maternal body burden; and finally, derivation of a daily intake by humans that would result in the tolerable human maternal body burden.

### **Calculation of body burden**

71. On the assumption that the critical period of exposure is GD16, the adverse effects following a single oral dose on GD15 would have been initiated at a time when the dose was undergoing tissue distribution. At this time, more of the maternal body burden would have been associated with well-perfused tissues, such as the liver, and the reproductive system and less with adipose tissue. It is possible to estimate the fetal exposure on GD16 by allowing for differences in the maternal dosage protocol using the toxicokinetic data of Hurst *et al*, following a single oral bolus dose on GD15<sup>27</sup> and following dietary administration of 1, 10 and 30ng/kg bw per day for 5 days per week from 13 weeks before mating<sup>11</sup>.

72. A problem with the interpretation of the Hurst *et al* papers<sup>11,27</sup>, which measured radioactivity after dosage with radioactive TCDD, is that the ratios of maternal to fetal body burdens on GD16 were not independent of dose, as would be predicted for such low doses. This non-linearity is difficult to explain on biological grounds and may have arisen as an artefact of the low levels of radioactivity measured. The SCF evaluation used regression analysis with a power model forced through the origin to correct maternal dosage and derive a correction factor of 2.6 for the higher fetal body burdens when dosed on GD15 compared with daily treatment.<sup>13</sup> These regressions used the ratios of maternal:fetal body burdens in ng/kg bw after single doses of 50 and 200ng/kg bw on GD15<sup>27</sup> (30:5.3 and 97.4:13.2, respectively) and after daily oral doses equivalent to 0.71, 7.1 and 21.3ng/kg bw/day<sup>11</sup> (20:1.4, 120:7.5 and 300:15.2 respectively). The JECFA evaluation confirmed the results of the power model but also used a linear model that gave a correction factor of 1.7, and the JECFA concluded that both models fitted equally well to the available data.<sup>14</sup> Although the power and linear models fitted equally well, they gave different correction factors, especially at very low body burdens. This resulted in a discrepancy (see JECFA, 2001)<sup>14</sup> when applied to the correction of the 5ng/kg bw subcutaneous maintenance dose used in the study of Faqi *et al*<sup>12</sup> (see below).

73. Because the correct mathematical model cannot be determined based on goodness of fit, and because the regressions are determined largely by body burdens higher than those relevant for derivation of a tolerable intake, we decided to adopt a simpler method of correction using the ratios calculated directly from the lowest doses in each of the studies by Hurst *et al*.<sup>11,27</sup> After a single oral dose of 50ng/kg bw on GD15, the fetal body burden on GD16 was 5.8-fold lower than the maternal body burden (5.3ng/kg bw compared with 30.6ng/kg bw).<sup>27</sup> After sub-chronic oral treatment with 1ng/kg bw/day for 5 days a week, which gave a maternal body burden of 19ng/kg bw, the fetal body burden on GD16 was 14.6-fold lower than the maternal body burden (1.3ng/kg bw compared with 19ng/kg bw).<sup>11</sup> Thus a bolus dose given on GD15 results in 2.5-fold higher fetal body burdens (14.6/5.8) on

GD16, than would occur if the same maternal body burden had arisen as a result of sub-chronic treatment.

### ***Derivation of the TDI***

74. In order to derive a tolerable intake for humans, it was necessary to convert the subcutaneous dosage regimen used in the Faqi study<sup>12</sup> into a steady-state maternal body burden on GD16. The study involved a bolus dose of 25ng/kg bw, 14 days before mating, and subsequent weekly maintenance doses of 5ng/kg bw. Assuming that the first day of mating corresponds to GD0, these weekly maintenance doses would have been given on GD-7, GD0, GD7, etc. By GD16, the doses given up to GD7 would have distributed to all tissues, representing steady-state distribution and resulting in a maternal body burden of 18.3ng/kg bw. This value is comprised of 9.3 + 2.3 + 3.0 + 3.7ng/kg bw remaining in the body from the doses given on GD-14, GD-7, GD0 and GD7, respectively, assuming a half-life of 21 days. The maternal body burden from the 5ng/kg bw maintenance dose given on GD14 would give a "non-equilibrium" maternal body burden of 4.5ng/kg bw on GD16. Using the correction factor described in paragraph 73, it can be estimated that a steady state maternal body burden of 2.5-fold higher (i.e. 11.3ng/kg bw) would be needed to produce the same fetal body burden as this "non-equilibrium" dose. Therefore the calculated total steady-state maternal body burden on GD16 arising from the subcutaneous dosing protocol at the LOAEL is approximately 30ng/kg bw, which would be about 33ng/kg bw after allowing for the TCDD intake from food.

75. Conversion of the calculated equivalent steady-state maternal body burdens from these studies in rats into an equivalent human body burden requires the use of uncertainty factors to allow for the use of a LOAEL and to allow for species differences and human variability. Both the SCF and the JECFA evaluations used a default factor of 3 to allow for the use of LOAEL, and an overall factor of 3.2 (10<sup>0.5</sup>) to allow for species differences and inter-individual variability.<sup>71,72</sup> The latter factor is lower than the default of 100 normally used because it incorporates the following chemical-specific adjustment factors:

- inter-species differences in toxicokinetics: uncertainty factor of 1.0 because the body burden approach allows for toxicokinetic differences;
- inter-species differences and human variability in toxicodynamics: uncertainty factor of 1 to cover both of these aspects based on the assumption that in general, rats are more sensitive than humans, but the most susceptible humans might be as sensitive to TCDD as rats;
- human variability in toxicokinetics: uncertainty factor of 3.2 to allow for potential increased accumulation, and hence body burden, of dioxins in the most susceptible individuals. This is only relevant for congeners with shorter half-lives than TCDD, because an individual with a 3.2-fold longer TCDD half-life would not reach steady-state body burden.

Applying the uncertainty factor of 9.6 (3 x 3.2) to the calculated maternal steady-state body burden from the study of Faqi *et al* (LOAEL=33ng/kg bw) gives a tolerable human equivalent maternal body burden of 3.4ng/kg bw.

76. Estimation of the daily intake of TCDD that would result in this body burden has to take into account the fraction absorbed (bioavailability) from the diet by humans (both the SCF and JECFA evaluations concluded that the bioavailability of

TCDD in humans is 50%), and the very long half-life in humans (which the JECFA concluded was an average of 7.6 years, while the SCF used a figure of 7.5 years). The human equivalent body burdens can be converted into daily intakes by the equation:-

$$\text{daily intake (pg/kg/day)} = \frac{\text{body burden (pg/kg bw)} \times \ln 2}{\text{bioavailability} \times \text{half-life in days}}$$

77. Using a bioavailability of 0.5 and a half-life of 2740 days (7.5 years), the tolerable human equivalent steady-state body burden from the study of Faqi *et al*<sup>12</sup> would be produced in humans by a daily intake of 1.7pg/kg bw/day. Given the imprecision and assumptions inherent in these calculations we concluded that the tolerable daily intake for dioxins and dioxin-like PCBs should be based on this value rounded to a single figure, i.e. 2pg WHO TEQ/kg bw per day. We note that SCF and JECFA have used longer averaging periods, but because intakes are usually expressed on a daily basis, we considered that establishment of a tolerable daily intake was more appropriate and transparent. This value is consistent with tolerable intakes derived recently using similar data (WHO: 1-4pg WHO TEQ /kg bw/day<sup>10</sup>; SCF: 14pg WHO TEQ /kg bw/week<sup>13</sup>; JECFA: 70pg WHO TEQ /kg bw/month<sup>14</sup>).

78. We note that the body burden is the most appropriate dose metric for establishment of a tolerable intake and, because of its long half-life, the body burden of TCDD at steady state is about 2000 fold higher the average daily intake. For example, an intake of 10 times the TDI on a single day would result in a 0.5% increase in the body burden. Therefore short term variation in intake does not significantly alter the body burden, and occasional exceedance of the TDI would not be expected to result in harmful effects, provided that intake averaged over a prolonged period is within the TDI.

## Conclusions

79. We *conclude* that dioxins and dioxin-like PCBs have the potential to cause a wide range of adverse health effects. The health effects most likely to be associated with low levels of exposures relate to the developing embryo/fetus.

80. We *recommend* that a tolerable daily intake of 2 pg WHO-TEQ/kg bw per day is established, based upon effects on the developing male reproductive system mediated via the maternal body burden.

81. We *consider* that this TDI is adequate to protect against other possible effects, such as cancer and cardiovascular effects.

82. We *note* that the most recent intake estimates for the UK population are 1.8 pg/kg bw/day for the average consumer and 3.1 pg/kg bw/day for the 97.5 percentile consumer and that dietary intakes are decreasing.

83. There are no short-term measures that can be used to decrease the body burden of dioxins and dioxin-like PCBs in humans because of their long half-lives and widespread presence at low levels in food.

84. Similarly, because of the long half-life, short-term exceedances of the tolerable intake are not expected to result in adverse effects. Nevertheless, it is not possible to identify a duration and degree of exceedance at which adverse effects might occur.

85. Finally, we *confirm* our previous advice that, although intakes of dioxins and dioxin-like PCBs by breast-fed babies are higher than is desirable, encouragement of breast-feeding should continue on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant.

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**COT/2001/07**

## **References**

1. Food Standards Agency (2000) COT statement on dietary exposure to dioxins and dioxin-like PCBs
2. Department of Health (1997) COT statement on the health hazards of polychlorinated biphenyls
3. Department of the Environment (1989) Dioxins in the Environment. *Pollution Paper No. 27*
4. Ministry of Agriculture, Fisheries and Food (1992). Dioxins in food. *Food Surveillance Paper No.31*
5. Department of Health 1993 Annual Report of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.
6. Department of Health (1995) COT statement on the US EPA draft health assessment document for TCDD and related compounds.
7. Department of Health (1999) COT statement on surveillance for PCDDs, PCDFs and PCBs in marine fish and fish products
8. Food Standards Agency (2000) COT statement on dioxins and dioxin-like PCBs in duck eggs
9. World Health Organization. Regional Office for Europe (1991). Summary report. Consultation on Tolerable Daily Intake from food of PCDDs and PCDFs. Bilthoven. Netherlands. 4-7 December 1990. **EUR/ICP/PCS 030(s) 0369n**. publ. WHO Regional Office for Europe. Copenhagen.
10. Leeuwen, F X R and Younes, M M (2000). Assessment of the health risk of dioxins: re-evaluation of the tolerable daily intake (TDI). *Food Additives and Contaminants*, **17**(4).
11. Hurst C H, DeVito M J & Birnbaum L S (2000); Tissue disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing Long-Evans rats following subchronic exposure; *Toxicol Sci* **57**:275-283
12. Faqi A S, Dalsenter P R, Merker H J & Chahoud I (1998) Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male

offspring of rats exposed throughout pregnancy and lactation. *Toxicol Appl Pharmacol* **150**:383-392

13. SCF (2001) Opinion of the Scientific Committee on Food on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food, 30 May 2001. Available at [http://europa.eu.int/comm/food/fs/sc/scf/outcome\\_en.html](http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html).
14. JECFA (2001). Joint FAO/WHO Expert Committee on Food Additives, Fifty-seventh meeting, Rome, 5-14 June 2001. Summary and Conclusions. Available at <http://www.who.int/pcs/jecfa/jecfa.htm>.
15. Ohsako S, Miyabara Y, Nishimura N, Kurosawa S, Sakaue M, Ishimura R, Sato M, Takeda K, Aoki Y, Sone H, Tohyama C and Yonemoto J (2001). Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5 $\alpha$ -reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. *Toxicol. Sci.* **60**: 132-143.
16. EPA (2000). Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds, September 2000. Available at [www.epa.gov/ncea/pdfs/dioxin/dioxreass.htm](http://www.epa.gov/ncea/pdfs/dioxin/dioxreass.htm).
17. Department of Health (2001). COC statement on carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin.
18. Fisher, J M, Jones, K W and Whitlock, J P, Jr. (1989). Activation of transcription as a general mechanism of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. *Mol. Carcinogen.* **1**: 216-221.
19. Hahn, M E (1998). The aryl hydrocarbon receptor: a comparative perspective. *Comp. Biochem. Physiol.* **121**: 23-53.
20. Huang, Z J, Edery, I, and Rosbash, M (1993). PAS is a dimerization domain common to *Drosophila* period and several transcription factors. *Nature* **364**: 259-262.
21. Mimura, J, Ema, M, Sogawa, K and Fujii-Kuriyama, Y (1999). Identification of a novel regulation of Ah (dioxin) receptor function. *Genes Dev.* **13**: 20-25.
22. Jain, S, Maltepe, E, Lu, M M, Simon, C and Bradfield, C A (1998). Expression of ARNT, ARNT2, HIF1a, HIF2a and Ah receptor mRNAs in the developing mouse. *Mech. Dev.* **73**: 117-123.
23. Poland, A and Knutson, J C (1982). 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related aromatic hydrocarbons: examination of the mechanism of toxicity. *Ann. Rev. Pharmacol. Toxicol.* **22**: 517-554.
24. Tomita, S, Sinal, C J, Yim, S H and Gonzalez, F J (2000). Conditional disruption of the aryl hydrocarbon receptor nuclear translocator (ARNT) gene leads to loss of target gene induction by the aryl hydrocarbon receptor and hypoxia-inducible factor 1 $\alpha$ . *Mol. Endocrinol.* **14**: 1674-1681.
25. Ema, M, Ohe, N, Suzuki, M, Mimura, J, Sogawa, K, Ikawa, S. and Fujii-Kuriyama, Y (1994). Dioxin-binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. *J. Biol. Chem.* **269**: 27337-27343.
26. Smart J and Daly A K. (2000). Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics* **10**: 11-24
27. Hurst C H, De Vito M J, Setzer RW and Birnbaum L. (2000a). Acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in pregnant Long Evans rats: Association of measured tissue concentrations with developmental effects. *Toxicol. Sci.*, **53**, 411-420.

28. Pohjanvirta, R, Vartiainen, T, Uusi-Rauva, A, Monkkonen J, and Tuomisto J. (1990). Tissue distribution, metabolism, and excretion of <sup>14</sup>C-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain. *Pharmacol Toxicol* . **66**: 93-100
29. Mason G and Safe S (1986). Synthesis, biologic and toxic properties of 2,3,7,8-TCDD metabolites. *Chemosphere*. **15**: 2081-2083.
30. Birnbaum L S (1986). Distribution and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin in congenic strains of mice which differ at the Ah locus. *Drug Metab. Disp.* **14**: 34-40.
31. Olson J R (1986). Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin in guinea pigs. *Toxicol. Appl. Pharmacol.* **85**: 263-273
32. Michalek J E and Tripathi R C (1999). Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 15-year follow-up. *J. Toxicol. Environ. Health, Part A.* **57**: 369-378.
33. Wolfe W H, Michalek J E, Miner J C, Pirkle JL, Caudill SP, Patterson DG Jr, Needham LL. (1994). Determinants of TCDD half-life in veterans of Operation Ranch Hand. *J Toxicol Environ Health* **41**: 481-8.
34. Furst P, Kruger C, Meemken HA and Groebel W (1989). PCDD and PCDF levels in human milk – dependence on the period of lactation. *Chemosphere* **18**: 439-444.
35. Roegner R H, Grubbs W D, Lustik M B, et al (1991). Air Force Health Study: an epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Serum dioxin analysis of 1987 examination results. NTIS# AD A-237-516 through AD A-237-524.
36. Grubbs W D, Wolfe W H, Michalek J E et al. (1995). Air Force Health Study: an epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Report No AL-TR-920107.
37. Webb K B, Evans RG, Knutsen AP, Roodman ST, Roberts DW, Schramm WF, Gibson BB, Andrews JS Jr, Needham LL and Patterson DG (1989). Medical evaluation of subjects with known body levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Toxicol Environ Health* **28**: 183-193
38. Ott M G, Messerer P and Zober A (1993). Assessment of past occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin using blood lipid analysis. *Int Arch Occup Environ Health* **65**: 1-8.
39. Flesch-Janys D, Berger J, Gurn P, Manz A, Nagel S, Waltsgott H, Dwyer JH (1995). Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. *Am J Epidemiol* **142**: 1165-1175.
40. Flesch-Janys D, Steindorf K, Gurn P, & Becher H (1998). Estimation of the cumulated exposure to polychlorinated dibenzo-p-dioxins/furans and standardised mortality ratio analysis of cancer mortality by dose in an occupationally exposed cohort. *Environ Health Perspect* **106 (suppl 2)**: 655-662
41. Piacitelli L A, Sweeney M H, Patterson D G Turner W E, Connally L B, Wille K K and Tompkins B (1992). Serum levels of 2,3,7,8-substituted PCDDs and PCDFs among workers exposed to 2,3,7,8-TCDD contaminated chemicals. *Chemosphere* **25**: 251-254
42. Steenland K, Piacitelli L, Deddens J, Fingerhut M & Chang L I (1999). Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-dioxin. *J Nat Cancer Ins* **91**:779-786
43. Sweeney MH, Fingerhut MA, Patterson D G jr, Connally LB, Piacitelli LA, Morris JA, Greife AL, Hornung RW, Marlow DA et al. (1990). Comparison of serum levels of 2,3,7,8-

TCDD in TCP production workers and in an unexposed comparison group. *Chemosphere* **20**: 993-1000.

44. Hooiveld M, Heerderik D J J, Kogevinas M, Boffetta P, Needham L L, Patterson D G Jr & Bueno-de-Mesquita H B (1998). Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols and contaminants. *Am J Epidemiol* **147**:891-901
45. Koopman-Esseboom C, Morse D C, Weisglas-Kuperus N, Lutkeschipholt I J, van der Paauw C G, Tuinstra L G M Th, Brouwer A & Sauer P J J (1994). Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatric Research* **36**:468-473
46. Pluim H J, Koppe J G, Olie K, van der Slikke J W, Slot P C & van Boxtel C J (1994). Clinical laboratory manifestations of exposure to background levels of dioxins in the perinatal period. *Acta Paediatr* **83**:583-587
47. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw C G, Tuinstra L G M Th, Weisglas-Kuperus N, Sauer P J J, Touwen B C L & Boersma E R (1995a). Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. *Early Human Development* **41**:111-127
48. Huisman M, Koopman-Esseboom C, Lanting C I, van der Paauw C G, Tuinstra L G M Th, Fidler V, Weisglas-Kuperus N, Sauer P J J, Boersma E R & Touwen B C L. (1995b). Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. *Early Human Development* **43**:165-176
49. Ilsen A, Briet J M, Koppe J G, Pluim H J & Oosting J (1996). Signs of enhanced neuromotor maturation in children due to perinatal load with background levels of dioxins. Follow-up until age 2 years and 7 months. *Chemosphere* **33**:1317-1326
50. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder M A J, van der Paauw C G, Tuinstra L G M Th & Sauer P J J (1996). Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *Pediatrics* **97**:700-706
51. Patandin S, Koopman-Esseboom C, De Ridder M A J, Weisglas-Kuperus N & Sauer P J J (1998). Effects of dioxins and polychlorinated biphenyls on birth size and growth in Dutch children. *Pediatric Research* **44**:538-545
52. Patandin S, Koopman-Esseboom C, De Ridder M A J, Sauer P J J & Weisglas-Kuperus N (1999). Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. *J Pediatrics* **134**:33-41
53. Pluim H J, van der Goot M, Olie K, van der Slikke J W, & Koppe J G (1996). Missing effects of background dioxin exposure on development of breast-fed infants during the first half year of life. *Chemosphere* **33**:1307-1315
54. Mocarelli P, Gerthoux P M, Ferrari E, Patterson DG Jr, Kleszak S M, Brambilla P, Vincoli N, Signorini S, Tramacere P, Carreri V, Sampson E J & Turner W E (2000). Paternal concentrations of dioxin and sex ratio of offspring. *Lancet* **355**:1858-1863
55. Eskenazi B, Mocarelli P, Warner M, Samuels S, Patterson D, Vercellini P, Olive D, Needham L & Brambilla P (2000). Seveso Women's Health Study: a study of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on reproductive health. *Chemosphere* **40**:1247-1253
56. Weisglas-Kuperus N, Patandin S, Berbers G A M, Sas T C J, Mulder PGH, Sauer P J J and Hooijkaas H (2000). Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ Health Perspect* **108**: 1203-1207

57. IARC (1997). *IARC Monographs on evaluation of carcinogenic risk to humans. Vol. 69.* Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans, Lyon, France: IARC.
58. Department of Health (1998). COC statement on 2,3,7,8-tetrachlorodibenzo-p-dioxin: Consideration of 1997 IARC monograph.
59. Bertazzi PA, Consonni D, Bachetti S, Rubagotti M, Baccarelli A, Zocchetti C and Pesatori A C (2001). Health effects of dioxin exposure: A 20-year mortality study. *Am J Epidemiol* **153**:1031-1047.
60. Burleson G R, Lebrec H, Yang Y G, Ibanes J D, Pennington K N and Birnbaum L S (1996). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on influenza virus host resistance in mice. *Fundam. Appl. Toxicol.* **29**: 40-47
61. Gehrs B C, Riddle M M, Williams W C and Smialowicz R J (1997). Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. II. Effects on the pup and the adult. *Toxicology* **122**:229-240.
62. Gehrs B C and Smialowicz R J (1999). Persistent suppression of delayed-type hypersensitivity in adult F344 rats after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology* **134**:79-88
63. Murray, F.J., Smith, F.A., Nitschke, K.D., Humiston, C.G., Kociba, R.J., and Schwetz, B.A. (1979). Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. *Toxicol. Appl. Pharmacol.* **50**:241-252.
64. Rier, S.E., Martin, D.C., Bowman, R.E., Dmowski, W.P., and Becker, J.L. (1993). Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam. Appl. Toxicol.*, **21**:433-441.
65. Rier, S.E., Turner, W.E., Martin, D.C., Morris, R., Lucier, G.W., and Clark, G.C. (2001). Serum levels of TCDD and dioxin-like chemicals in rhesus monkeys chronically exposed to dioxin: correlation of increased serum PCB levels with endometriosis. *Toxicol. Sci.*, **59**:147-159.
66. Kociba R J, Keyes D G, Beyer J E, Carreon R M, Wade C E, Dittenber D A, Kalnins R P, Frauson L E, Park C N, Barnard S D, Hummel R A and Humiston C G (1978). Results of a two-year chronic toxicity and oncogenicity study of 2378TCDD in rats. *Toxicol Appl Pharmacol* **46**:279-303.
67. Hassoun EA, Wilt SC, Devito MJ, Van Birgelen A, Alsharif NZ, Birnbaum LS, Stohs SJ. (1998). Induction of oxidative stress in brain tissues of mice after subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Sci.* **42**:23-7.
68. Gray, L.E. Jr., Ostby, J.S., and Kelce, W.R. (1997). A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male Long Evans Hooded rat offspring. *Toxicol. Appl. Pharmacol.*, **146**:11-20.
69. Gray, L.E. Jr., Kelce W R, Monosson E, Ostby, J S and Birnbaum L S (1995). Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicol. Appl. Pharmacol.*, **131**:108-118.
70. Mably, T.A., Bjerke, D.L., Moore, R.W., Gendron-Fitzpatrick, A., and Peterson, R.E. (1992). In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductive capability. *Toxicol. Appl. Pharmacol.*, **114**:118-126.

71. IPCS (1994) Environmental Health Criteria **170**: Assessing Human Health Risks to Chemicals. Geneva, World Health Organisation, International Programme on Chemical Safety.
72. IPCS (1999) Environmental Health Criteria **210**: Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. Geneva, World Health Organisation, International Programme on Chemical Safety.

Lay summary of the COT statement on the tolerable daily intake for Dioxins and Dioxins-like Polychlorinated Biphenyls

Consideration of the TDI for Dioxins and Dioxins-like PCBS: Evaluation of exposure data in the epidemiological studies