

Comments on the

**“Opinion of the Scientific Committee on Food on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food. Update based on new scientific information available since the adoption of the SCF Opinion of 22 November 2000”
Adopted on 30 May 2001
(CS/CNTM/DIOXIN/20 final)**

by the German Federal Environmental Agency
(Umweltbundesamt)
Update 20 December 2002 based on comments from 20 December 2001

1. The re-assessment of the SCF

The Scientific Committee on Food decided to review its recommendation on the acceptable daily intake of dioxins (and dioxin-like PCB), only half a year after the original recommendation was made in November 2000. In the paper of May 2001 the SCF mentioned: “The Committee is asked to consider whether there is a need to update its opinion on the risk assessment of dioxins and dioxin-like PCBs in food on the basis of new scientific information available since the release of the SCF opinion of 22 November 2000.” And: “In addition, the SCF took cognisance of comments received from the Swedish National Food Administration (2001), the Norwegian Food Control Authority (2001) and from some members of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) of the European Commission.” The fact that the SCF made transparent neither by whom it was asked to review its first paper nor that it had published the comments made by member states and CSTEE members (SCF, 2001) makes it more difficult to comment on this paper.

As a result of this review the SCF recommended a tolerable daily intake of 2 pg/kg bw per day (or 14 pg/kg bw per week to express that the long term exposure is relevant) doubling the TDI proposed some months earlier by the same committee (SCF, 2000).

Potential health effects of dioxins are an important issue of concern to both politicians and the public. As all humans are exposed to measurable levels of dioxins and related substances, it is a very significant decision to elevate the level of the tolerated daily intake. Moreover the revision of this value is not only of academic interest but may also directly influence limit values guiding

risk reduction measures, such as those for tolerated residues in food. Thus the level of the TDI may indirectly influence entire sectors of industry in some member countries such as the fish industry of some Nordic States or the feed industry throughout the European Union.

It has to be borne in mind that this report also forms a standard for further risk assessments of other substances.

Because of the severe consequences of the recommendation, the possible lowering of the level of protection of humans, and the unusually fast revision of the former recommendation, the daily intake level the SCF has decided to be acceptable should be expected to involve a very low likelihood of a risk for humans.

In particular some prerequisites have to be fulfilled by a report lowering the level of human protection:

- The proposed TDI has to protect all subpopulations. In the case of dioxin this is of high importance as the exposure of infants through breast feeding may exceed the exposure of adults by one or two orders of magnitude.
- The degree of uncertainty should be indicated at every step of the risk assessment as requested by the Communication from the Commission on the Precautionary Principle *“Where possible, a report should be made which indicates the assessment of the existing knowledge and the available information, providing the views of the scientists on the reliability of the assessment as well as on the remaining uncertainties. If necessary, it should also contain the identification of topics for further scientific research ”* Uncertainty of the models applied should be characterised carefully and assumptions made should be checked for their plausibility (Commission of the European Communities, 2000).
- Epidemiological findings should support the risk assessment based on animal experiments.
- Recent scientific papers published after the SCF opinion should support the risk assessment made.

After carefully studying the SCF opinion of May 2001, it remains doubtful whether it fulfils the above mentioned criteria.

2. The scientific discussion on TDI for dioxin-like substances

Nearly all human health risk assessments carried out on dioxins (PCDD/PCDF and dioxin-like PCBs) in the past (with the exception of the one by US-EPA) recommended health based exposure limits within the range of 1-10 pg X/kg bw per day (X = I-TEQ (Nato-CCMS), Nordic-TEQ, BGA-TEQ and finally WHO-TEQ).

As early as 1985 the Federal Environmental Agency in Berlin (UBA) evaluated 2,3,7,8-TCDD together with the former Federal Health Office (UBA, 1985).

A tolerable daily intake of 1-10 pg I-TEQ/kg bw per day was recommended on the basis of a 2 year carcinogenicity study on rats (Kociba et al., 1978). The lower end of the range (1 pg I-TEQ/kg bw per day) has been used as a precautionary value, the higher end (10 pg I-TEQ/kg bw per day) as a level requiring regulatory action. These values have been derived from a NOEL of 1ng/kg bw for hepatocellular neoplasms in female SD rats, applying uncertainty factors of 100 – 1000.

In 1990 the WHO European Center of Environment and Health (WHO-ECEH) established a TDI of 10 pg/kg bw per day for TCDD (WHO, 1991). This value was based on a pharmacokinetically derived NOAEL of 100 pg/kg bw per day for humans and the application of an uncertainty factor of 10 for intraspecies variation.

In 1996 the Committee on the Risk Evaluation of Substances of the Health Council of the Netherlands recommended a health based exposure limit of 1 pg TEQ_{Total}(PCDD/PCDF/PCB)/kg bw per day (Health Council of the Netherlands, 1996).

This was the first evaluation which included dioxin-like PCBs in addition to PCDDs and PCDFs.

As the most relevant studies to derive a health based recommended exposure limit, chronic feeding studies on rhesus monkeys and marmoset monkeys were identified.

Toxicological endpoints used were neurobehavioral effects in baby rhesus monkeys (Bowman et al., 1989) and effects on the immune system (change in lymphocytes) of marmosets (Neubert et al., 1992). From these studies a LOAEL of 100 pg TCDD/kg bw was determined. Uncertainty factors of 2 for the extrapolation to a NOAEL, 5 for interspecies (monkey – human)

variation and 10 for intraspecies variation (human) were applied to the LOAEL resulting in an exposure limit of 1 pg TEQ_{Total}/kg bw per day.

This exposure limit was counterchecked with the results of epidemiological studies on infants in the Netherlands. Infants from mothers exposed to a median daily dose of 3 pg TEQ (PCDD/PCDF/dioxin-like-PCB)/kg bw per day showed slight neurological effects, changes in the hormone status and in immunological functions (Huisman et al., 1995; Koopman-Esseboom et al., 1994; Weisglas-Kuperus et al., 1995) This gave the Committee further support for the recommended exposure limit of 1 pg TEQ/kg bw per day.

Following an expert meeting in Stockholm in 1997, organized by the WHO, where consensus toxic equivalency factors (TEFs) for PCDDs, PCDFs and dioxin-like-PCBs were derived for humans, fish and wildlife (Van den Berg et al., 1998), an expert consultation under the umbrella of WHO-ECEH and IPCS was organized in May 1998 in Geneva.

The objective of this expert meeting was the health risk assessment of dioxins, with a view to establishing an updated TDI for dioxins.

The expert consultation ended with a recommendation for a TDI- value of 1-4 pg WHO-TEQ/kg bw per day (WHO, 2000).

The basis of this evaluation were TCDD-feeding studies on rhesus monkeys (endometriosis, neurobehavioral effects in offspring) and single dose application to pregnant rats (Long Evans, Holtzman, F344) on GD14/GD15 by gavage (sperm count-,genital malformation- and immune suppression in offspring). The dose related estimates of body burdens of the experimental animals were used for the calculation of 'estimated daily intakes' (EDI) for humans.

In its executive summary (WHO, 2000), the WHO-consultation recognized that "certain subtle effects may be occurring in some sections of the general populations of industrialized countries at current intake levels (2-6 TEQ pg/kg bw per day) and body burdens (4-12 TEQ ng/kg bw),...".The consultation also stressed that "the ultimate goal is to reduce human intake levels below 1 pg TEQ/kg bw per day".

In November 2000 the Scientific Committee on Food of the European Commission published an 'Opinion of the SCF on the Risk assessment of Dioxins and Dioxin-like PCBs in Food' (SCF, 2000).

On the basis of this extensive review of data and experimental results the Committee recommended a temporary tolerable weekly intake (t-TWI) of 7 pg WHO-TEQ/kg bw per week.

The evaluation was similar to the WHO approach, using slightly different estimates of body burdens of the animals investigated. The 'key-studies' selected for the evaluation of a tolerable intake were nearly the same as used by the WHO evaluation. Because of the mentioned slight differences of the estimates of the body burdens of the experimental animals, the SCF suggested a tolerable intake in the range of 1-3 pg WHO-TEQ/kg bw per day. As in the opinion of the Committee no scientific data were available to give preference to a single value out of the range of 1-3 pg WHO-TEQ/kg bw per day, it decided to recommend 1 pg WHO-TEQ/kg bw per day as a temporary tolerable intake, taking into account the uncertainties of the evaluation. Because of the long half-lives of dioxins in humans (7 years or more) The committee decided to establish a temporary intake on a weekly basis of 7 pg WHO-TEQ/kg bw per week.

Only six months later the SCF carried out a re-evaluation of its t-TWI from November 2000 (SCF, 2001).

With reference to new scientific information on the toxicity of dioxins, comments of two national food administrations and comments from members of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) of the European Commission (to none of which reference was made in the re-evaluation), the SCF found it 'appropriate to revisit and update its assessment'.

The SCF regarded - in contrast to its evaluation of November 2000 - the long-term feeding studies on rhesus monkeys (Schantz and Bowman, 1989 and Rier et al., 1993) as well as the studies of Gehrs et al. (1997) and Gehrs and Smialowicz (1998) no longer as 'pivotal studies'. As 'new' pivotal studies it identified a study by Faqi (Faqi et al., 1998) and a study by Ohsako (Ohsako et al., 2001). This reconsideration of 'pivotal studies' led to the situation that the re-assessment is now based only on rat studies which investigated only reproductive effects only on male offspring and, in addition, three of these studies are single dose studies at gestational day 15 (GD15).

From these studies LOAELs were derived, maternal steady state body burden estimated - using data from Hurst et al. (2000a and 2000b) - and finally the associated estimated human daily intakes (EHDI) calculated. Applying an overall uncertainty factor of 10 to the LOAEL derived EHDI, the SCF

concluded (on the basis of the Faqi-study) “that 2 pg/kg bw per day should be considered as a tolerable intake for 2,3,7,8-TCDD”.

Again reference was made to the long half-lives of dioxins in humans to establish a TWI of 14 pg WHO-TEQ/kg bw per week

The SCF stressed the fact that a considerable proportion of the European population would still exceed the TWI set. Therefore, “continuous efforts should be made to limit environmental release of PCDDs, PCDFs and dioxin-related compounds.”

Similarly the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated PCDDs/PCDFs and dioxin-like PCBs at its 57th Meeting (JECFA, 2001).

In the summary of this meeting, which is in part identical with the SCF- re-evaluation from May 2001, JECFA recommended a provisional tolerable monthly intake (PTMI) of 70 pg WHO-TEQ/kg bw per month, which is the mean of a calculated range of 40-100 pg WHO-TEQ/kg bw per month.

Overview of recently recommended limit values for PCDDs, PCDFs and dioxin-like PCBs using WHO-TEFs:

WHO (1998) : TDI of 1-4 pg WHO TEQ/kg bw per day

SCF (2000) : t-TWI of 7 pg WHO TEQ/kg bw per week

SCF (2001) : TWI of 14 pg WHO TEQ/kg bw per week

JECFA (2001) : PTMI of 70 pg WHO TEQ/kg bw per month

This overview of limit values for dioxins and dioxin-like PCBs has the common feature that the values were derived from non carcinogenic endpoints and the confusing feature that they refer to different exposure periods.

Expressed as daily intake all these recommendations are between a range of 1-4 pg WHO TEQ/kg bw per day.

Following the WHO re-evaluation in 1998, the Federal Environmental Agency in Berlin consulted experts in 1999 to discuss the recommended TDI of 1-4 pg WHO-TEQ/kg bw per day in relation to its own evaluation from 1985 (1-10 pg TCDD/kg bw per day) and the later (BGA, 1993) accepted range of 1-10 pg TEQ(NATO-CCMS)/kg bw per day.

3. German position on TDI

The WHO toxic equivalency factors (WHO-TEFs) derived at an expert meeting in Stockholm in 1997 (Van den Berg et al., 1998) were accepted and recommended for use for future risk assessments in Germany.

The re-evaluation of the TDI for dioxins and dioxin-like PCBs in 1998 (WHO, 2000) was acknowledged, although the uncertainty factor of 10 applied in the extrapolation of the LOAELs in experimental animals to NOELs in humans was regarded as to be too small. Therefore it was recommended to use the lower end value of 1 pg WHO-TEQ/kg bw per day, the former precautionary value in Germany, as the TDI.

It was also stated that the existing exposure of nursed babies of about 100 pg WHO-TEQ/kg bw per day is too high and urgently needs to be further reduced.

Last but not least it was proposed that the new precautionary value should be well under 1 pg WHO-TEQ/kg bw per day.

Based on these decisions Germany welcomed the SCF recommendation of November 2000 with its t-TWI of 7 pg WHO-TEQ/kg bw per week, which is equivalent to a TDI of 1 pg WHO-TEQ/kg bw per day.

4. Evaluation of the Opinion of the SCF from May 2001

4.1 General comments

The re-evaluation of the SCF from May 2001 amends the previous evaluation and further specifies it, based on mathematical calculations, without taking adequately into account the uncertainties and/or the variations of the underlying assumptions.

The core of the re-evaluation is the choice of 'pivotal' studies. All results used are from 4 studies on male offspring of pregnant rats, administered single doses by gavage (except the study of Faqi) on gestation day 15 and using effects on the reproductive system as the most sensitive effects. In other words, the re-evaluation is mainly based on one effect in one species, on one gender and a single dose.

These restrictions may be advantageous for getting more precise numbers as a result of the evaluation, but may be disadvantageous in the overall evalua-

tion with respect to the variety of sensitive effects seen in the genetically widely variable human population with life long exposure.

The former evaluation of the SCF and the WHO included studies on rhesus monkeys and covered also immunological and neurotoxicological effects, which are thought to be of high relevance for men.

In general the database for the evaluation of a health based limit value (or a range of values) is much broader than the database used in the re-evaluation and is more representative for the multitude of effects and exposure in humans.

4.2 Can the proposed TDI protect all relevant subpopulations of the human society?¹

Human exposure to dioxins and related compounds has been the subject of various international studies conducted during the past few years (e.g. SCOOP, 2000, AEA, 1999, EPET, 2002). More than 90 percent of human dioxin exposure derive from food and 80 percent are from food of animal origin, 5 to 10 percent of intake come from soil, air, textiles and unknown sources.

In industrialised countries the average daily intake of dioxin-like substances is between 1 and 3 pg / KG bw per day (Patandin et al., 1999; Tsutsumi et al., 2001, Schechter et al., 2001). The differences in the uptake of these substances between adults in different countries are relatively small and within the range of 1 – 4 pg/kg x day proposed as TDI by the WHO. The daily intake of PCDD/F for German adults was 2 pg TEQ/kg bw per day in 1985 (Fürst, 1987) and decreased to 1,2 pg TEQ/kg bw per day in 1995 (Fürst et al., 1997). The average dietary intake of PCDD/F for adults in 8 European countries varies from 0.5 (Norway) to 1.7 pg TEQ/day (France) per kg body weight and day.

The contribution of dioxin-like PCBs to the total WHO-TEQ is estimated to be about 50 percent for most foods with the exception of fish which can be more than 80 percent of the total TEQ (SCOOP, 2000). The total dietary intake of PCB TEQ ranges from 0.8 to 1.8 pg WHO TEQ per day. That means that the overall dietary intake of dioxins and dioxin-like PCBs ranges from 1.6 to 2.6 pg TEQ/kg bw (SCOOP, 2000; Focant et al., 2002; Liem et al., 1997; Cuervo et al., 2002).

¹ For better comparability in the following text I-TEQ are converted to WHO-TEQ by adding 15 percent (EPA 2000).

In contrast, there are tremendous differences in uptake rates between humans of different ages. As children consume more food than adults in relation to their body weight, and have other consumption habits, children ingest higher doses of dioxin-like compounds than adults. The dose per unit body weight for children decreases with increasing age, but the daily dose (pg/day) increases with age and remains more or less constant in adults over 20 years of age (WHO, 2000). Patandin et al. (1999a) found a mean daily intake of dioxin-like compounds in young Dutch adults (20-25 years) of 2.3 and 2.0 pg TEQ/kg bw per day for males and females respectively. In the groups of 1 – 5 year old children and children between 6 and 10 years of age, the mean daily intake was still two to three times higher than in young adults. Similar results were described by AEA (1999) for UK and Wittsiepe et al. (2001) in a German duplicate study with children aged 14 to 47 months (Umweltbundesamt, 1999).

The SCF (2000) stated that 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. This corresponds with the data from Patandin et al. (1999a) indicating a daily intake in breast fed infants up to six months of 112 to 118 pg TEQ/kg bw. The US figures are similar (Schechter et al., 2001) with 2.2 – 2.4 pg/kg bw per day for female and male adults and 42 pg/kg bw per day for breast fed infants. Recent data for Germany show that a 4 month old infant has an intake of 66 pg TEQ/kg bw per day via human milk (BMU, 2002). This is about 50-60 times the intake of an adult.

Uncertainties remain about whether the high perinatal exposure of infants leads to a reservoir that is at least partially maintained until puberty (La Kind an Filser, 1999; Patandin et al., 1999b). Most probably the question whether dioxin-like substances from early exposure persist until puberty can only be decided on the basis of the toxicokinetics of every congener contributing to the overall TEQ load as half lives of substances differ significantly and also differ with age (Chen, 2001). For example the 2,3,4,7,8 PeCDF-congener contributed about 40 percent of the TEQ for dioxins with an estimated half life of 19.6 years (EPA, 2000, Flesch-Janys et al., 1996). Recent German data suggest that the body burden of formerly breast-fed children aged 9-11 is still 20 percent higher than those of their formula-fed age-mates (BMU, 2002).

This means in consequence that toleration of a daily intake for adults of 2 pg/kg bw per d for the general adult population means accepting at the same time that this limit is exceeded more than 50 fold in young children.

Dioxin-like compounds are rapidly absorbed into the body and slowly eliminated. Therefore the body burden is a reliable indicator of time-integrated exposure and absorbed dose (EPA, 2000). Uncertainties for the calculation of the body burden are the absorption rate, distribution in the body and the half-life of the congeners. Blood dioxin levels on lipid basis are often used to estimate body burden or exposure. The level of dioxins, dibenzofurans and dioxin-like PCBs may vary in different tissues (Schechter et al., 1998), but in adipose tissue and blood the levels are almost similar when presented on a lipid basis (Iida et al., 1999).

The median dioxin level for German adults is 18 pg TEQ/g fat in blood and the annual increase in dioxin concentration in the body of adults was estimated to be 0.3 pg TEQ/g lipid (Päpke et al., 1997). The corresponding body burden is 3.6 to 5.4 ng TEQ/kg bw calculated on a lipid content of the body of 20 to 30 percent.

The body burden of dioxin-like compounds in pregnant women will result in the exposure of the foetus because these contaminants cross the placenta. Kreuzer et al. (1997) found in adipose tissues of 3 stillborns PCDD/F TEQ-levels ranging from 11.2 to 12.4 pg/g lipid with a TCDD burden of 1.3-2.1 pg/g lipids.

Schechter et al. (1998) analysed tissues from 5 American mothers and their infants. The mean level in cord blood for PCDD/Fs and coplanar PCBs was 6.5 pg TEQ/g and thus about half the level in the mother's placenta (11.9) and blood (13.6) on lipid base. Wang et al (2002) and Abraham et al. (1998) obtained similar results. Assuming the lipid concentration is the same in mother and child the body burden of the newborn is half the body burden of the mother. This contrasts with the maternal/foetal quotient calculated by the SCF (12.3 ng/kg bw for the dams corresponds to 1.2 ng/kg bw for the foetal body, one tenth).

After at least 17 weeks nursing the blood dioxin level of the 12 month old infants were about two to three times higher than the blood dioxin levels of their mothers (Abraham et al., 1998).

All newborns are subject to prenatal exposure via placenta, a at least 50 fold higher intake by nursing and a two to three times higher dietary intake on body weight until at least the age of 10 years. In order to protect the whole population it is essential to include the vulnerable group of children in the risk assessment process.

There are two possible ways to reduce the extremely high toxic load in breast fed infants. The first possibility is to formula feed the infants, as formula fed infants have much lower dioxin intake than their breast fed contemporaries. As breast feeding has measurable benefits for neurological and immunological development, formula feeding can not be recommended as a substitute for breast feeding. The only remaining way to lower the dioxin uptake is to drastically reduce the background exposure of the general population.

4.3 Is the degree of certainty of the assessment properly indicated by the SCF?

The re-assessment of the SCF is based on a number of assumptions the committee made. These assumptions involve some uncertainties that are not sufficiently reflected in the paper.

The core of the re-evaluation is the transformation of single dose, as administered in the pivotal studies, to a subchronic exposure by comparing the resulting maternal and foetal body burdens at gestational day 16. As there were no data available for the mixture of substances contributing to the overall TEQ body burden the SCF used the data provided by Hurst et al. (2000 a,b).

The SCF also transformed the weekly maintenance dose of 5 ng/kg bw administered in the Faqi study (Faqi et al., 1998). The assumption that this dose is to be considered an additional acute dose appears doubtful. The exposed individuals are likely to have already reacted to the preceding doses (e.g. enzyme induction); secondly, the dose was administered on GD 14, whereas the SCF puts the critical window for foetal exposure for reproductive endpoints at GD 16.

By choosing this procedure the SCF made the basic assumption that the body burden on GD 16 is the relevant body burden at the time when the effects under study are caused in the foetus. That implies firstly that the effect is induced at a single point of time and is not produced by a longer lasting perturbation of the foetal development and secondly that this effect is caused precisely on GD 16. As a matter of fact none of the pivotal studies indicate at which time of pre- or postnatal development the alterations are induced, finally leading to the expression of endpoints under study.

In calculating the associated estimated daily intake (EHDI), the SFC used 50 percent as the fraction of dose absorbed (for absorption from food for humans, SFC 2000), citing WHO 2000 (SCF 2001). The basis of this assumed fraction of dose absorbed from human food is unknown. Studies indicate

however a significantly higher absorption rate (Poiger and Schlatter, 1986, 89 percent). It should also be considered that infants and children absorb higher rates and that small doses are more readily and effectively absorbed (EPA, 2000). For breast fed infants the absorption of PCDDs, PCDFs and PCBs from human milk is about 90 percent, expressed in TEQs (Abraham et al., 1996).

The influence of an absorption factor of 0,8 (for adults) on the calculated EHDIs by the SCF (SCF, 2001) can be shown by the application of a divisor of 1,6 ($f=0.8/f=0.5$). The resulting EHDIs will then be within the range of 6,2 – 30 pg/kg bw. Applying the same uncertainty factors as the SCF, one would end up with a tolerable daily intake of slightly above 1 pg/kg on the basis of the Faqi study (Faqi et al., 1998).

In addition, uncertainty is also inherent in the use of a half life of 7,5 years (2,3,7,8-TCDD) for the calculation of the EHDIs, because it does not take into account the real mixture of PCDD/PCDF in the food and in fatty tissue. Ideally an overall TEQ half life should be applied.

A first estimate of an overall TEQ half life using adipose tissue concentrations of PCDD/PCDF from breast-fed infants (Kreuzer, 1997) and half life (Flesch-Janis et al., 1996) for 2,3,7,8-TCDD, 1,2,3,7,8-Penta CDD, 1,2,3,5,7,8-Hexa CDD and 2,3,4,7,8-Penta CDF (which contribute 85 percent to the overall PCDD/F-TEQ in adipose tissue) leads to an overall TEQ half life of about 15,5 years. This calculated half life is very similar to the measured overall TEQ (PCDD/F, dioxin-like PCB) half life of 15 years in human milk samples (Norén and Meironyte, 2000). It is assumed, that the relative contribution of the selected congeners to the overall PCDD/F-TEQ in the adipose tissues from infants and adults is similar.

If this overall TEQ half life is used for the calculation of EHDIs an additional divisor of 2 (15years / 7.5years) has to be applied, that means that the range of the EHDIs would be 3 –15 pg/kg bw. When an uncertainty factor of 9.6, is applied to the calculated EHDIs, as used by the SCF in its re-evaluation from May 2001, a range between 0.3 and 1.5 pg/kg bw per day is obtained for the tolerable intake. Using the Faqi-study as the most relevant one (SCF, 2001), the resulting TDI would be 0.6 pg WHO TEQ/kg bw per day .

The examples given above show, that the application of measured absorption rates – instead of assumed ones - together with an overall TEQ half life, which reflects the real situation better than 2,3,7,8-TCDD alone, will shift the calculated TDI to levels below 1 pg/kg bw per day.

The choice of the “uncertainty” factor to account for the use of the LOAEL instead of NOAEL should more appropriately be based on the quotient LOAEL /NOAEL (50 : 12.5 or 79 : 19) determined in the Ohsako study (Ohsako et al., 2001), likewise in rats, and this factor would at least then be 4, because Ohsako also reported a significant decrease in androgen receptor (AR) mRNA level in the ventral prostate during the prepubertal period of the male offspring at the chosen NOAEL. In addition it is questionable whether the chosen LOAELs are really LOAELs.

Mably concluded: ‘From the dose-response curve for cauda epididymal sperm number (Mably et al., 1992, Fig. 4) the LOAEL can be estimated to be substantially lower than 0.064 µg TCDD/kg (the lowest dose tested)’.

From the Gray study a LOAEL of 50ng/kg bw per day has been derived (Gray,1997). This dose was also the lowest dose tested. A NOAEL was not established experimentally. The dose response curve for ejaculated sperm numbers however indicates that there might be an effect at lower doses. A more detailed analysis of the dose response relationship, for instance with a benchmark procedure, could clarify the situation.

In addition, Gray stated in his conclusion that ‘fetal concentrations of 5 ppt TCDD are associated with accelerated eye opening and a 25% reduction in ejaculated sperm counts. The body burden (fetal concentration) of 5ng/kg bw corresponds with the LOAEL of 50 ng/kg bw per day. This fetal concentration is in the same range as the concentration found in stillborns (Kreuzer, 1997). This indicates that the margin of safety might be at least very small if it does exist at all.

Faqi (Faqi et al., 1998) came to the conclusion: ‘We have reported that the lowest dose tested (TCDD 25/5) produces a significant effect on sperm number, daily sperm production, and sperm morphology so that the LOAEL and the NOAEL can be regarded to be substantially lower (than the estimated daily dose of 0.8 ng/kg bw per day)’.

For extrapolation to humans, such information from experimental data is, in our view, to be preferred to default assumptions.

The decision not to apply an uncertainty factor for differences in toxicodynamics between experimental animals and humans and for inter-individual variation among humans is not comprehensible.

4.4 The ‘pivotal studies’

All 4 studies selected as pivotal studies (Mably et al., 1992; Gray et al., 1997; Faqi et al., 1998; Ohsako et al., 2001) are studies designed as developmental toxicity studies on male offspring of perinatally exposed pregnant rats. In three studies (Mably, Gray, Ohsako) the pregnant rats were exposed on GD15 to a single oral dose by gavage.

The assumption underlying the selected species and dose regimen is that ‘the male reproductive system of the rat is the most sensitive target of possible adverse effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (Faqi et al., 1998), and that the GD15 precedes the sensitive window for the induction of adverse effects in the male reproductive system.

The restrictions of the pivotal studies selected by the SCF also warrant comment with respect to:

- the restriction to one species
- the restriction to one gender
- the application route as relevant for the human exposure
- the duration of exposure (acute versus chronic)

Studies for immunological and behavioural effects as well as carcinogenicity studies have not been included as pivotal studies though the inclusion of their results would lead to considerably lower TDIs.

4.5 Developmental effects

The SCF excluded findings of endometriosis from the data it used for the derivation of ADI, although we believe they deserve more than minor acknowledgement. TCDD induced endometriosis not only in rhesus monkeys (Rier et al., 1993) but also in rats and mice (Cummings et al., 1996). Furthermore, a higher, nonsignificant risk for endometriosis was determined in an epidemiological study with the “Seveso cohort” (Eskenazi et al., 2002). In this historical Seveso cohort study, Eskenazi et al. (2002a) examined a suspected link between dioxin exposure and endometriosis. The cohort consisted of 97 women from Zone A (serum TCDD levels: median: 257 ppt; 25th percentile: 114 ppt; 75th percentile: 713 ppt) and 540 women from Zone B (serum TCDD levels: median: 47.0 ppt; 25th percentile: 22.5 ppt; 75th percentile: 220.0 ppt) who were 30 years old or younger at the time the disaster occurred. Endometriosis disease status was defined by pelvic examination, previous pelvic surgery and ultrasound. “Cases” were women who had surgi-

cally confirmed disease or an ultrasound consistent with endometriosis. “Nondiseased” women had surgery with no evidence of endometriosis or no signs or symptoms. The other women were classified as having “uncertain status”. 19 women with endometriosis and 277 nondiseased women were identified. The age-adjusted relative risk was 1.2 for a “20.1-100 ppt group” (n = 285, 90 % CI 0.3-4.5) and 2.1 for a “>100 ppt group” (n = 205, 90 % CI 0.5-8.0), relative to a group with serum TCDD levels \leq 20 ppt. The authors report a doubled, nonsignificant risk for endometriosis among women with serum TCDD levels of 100 ppt or higher. Birnbaum and Cummings (2002) see a plausible link between exposure to TCDD and endometriosis.

Among other epidemiological studies, those concerned with the sex ratio of offspring of parents exposed to dioxins are of relevance. A statistically significant increase in female births was found at higher concentrations with increasing serum TCDD levels in fathers or parents from Seveso (Mocarelli et al., 1996, 2000). Ryan et al. (2002a) observed a significant decrease in male births for workers exposed to pesticides (trichlorophenol and 2,4,5-trichlorophenoxy acetic acid) associated with high levels of dioxins. A trend towards more female births was also reported by Moshhammer and Neuberger (2000) for a cohort of workers of a chemical plant producing 2,4,5-trichlorophenol. Even if this effect could not be confirmed for workers of an Agent Orange production plant (Schnorr et al., 2001) or for Agent Orange users (Michalek et al., 1998), and was not found in studies with monkeys (Bowman et al., 1989) and rats (Faqi et al., 1998), a change in the sex ratio must be considered a very relevant effect and requires discussion as to whether it should be taken into account in the derivation of TDI values.

4.6 Behavioural effects

The work of Markowski et al. (2001) describes behavioural changes in the female offspring of Holtzman rats that were exposed to a single oral dose of TCDD at gestational day 18. The doses applied were 0, 20, 60, and 180 ng/kg. After postnatal day 77 the adult female offspring of the dosed rats were trained to respond on a lever for brief opportunities to run in a running wheel. Once they had begun responding on a fixed-ratio schedule of reinforcement, the fixed-ratio requirement for lever pressing was increased. Perinatal TCDD exposure produces a significant dose-related reduction in the number of opportunities to run, the lever response rate and the total number of revolutions in the wheel. Contrary to the pivotal studies used by the SCF, a consistent dose-response relationship could be observed, making it possible to calculate a dose response curve and to derive benchmark doses from it. The ED₀₁ for total wheel revolution was 0.71 ng/kg, the corresponding ED₁₀ was 7.32 ng/kg. In experiments with the offspring of Sprague-

Dawley rats that received a single TCDD-dose of 0, 20, 60, and 180 ng/kg at day 8 of gestation, Hojo et al. (2002) tested the influence on schedule controlled operant behaviour (SCOB) in both sexes of the offspring. TCDD evoked a sexual dimorphic response pattern. Treated males responded in general at lower rates, females at higher rates than the untreated respective controls. ED₀₁ for two measures of sexually dimorphic performance were 270 and 300 pg/kg with 95 % lower bounds of 180 and 130 pg/kg respectively.

These findings agree with findings of morphological brain changes such as a reduction of cortical thickness, the reversal of right hemispheric dominance to left hemispheric dominance in male offspring and a change of dominance from left to right in female offspring of rats treated with the same doses at GD 8 (Zareba et al., 2002). Taking the ED₀₁ as a surrogate for the NOAEL these results would lead to a much lower TDI than the pivotal studies used by the SCF. Though it is impossible to calculate an assumed steady state dose for this experiment as the body burden ratios are not known, these findings are clearly inconsistent with an elevated TDI as proposed by the SCF. The authors of the studies point out that the range of benchmark doses derived from the current dose-response data approaches or falls below the human background concentration estimated to be 13 ng/kg by deVito et al. (1995).

Interestingly in both studies U-shaped dose response curves were observed. This warrants a precautionary approach to be taken in the extrapolation of higher dose experiments to those low doses relevant for human exposure.

Though Markowski's (2001) work was published shortly after the SCF re-evaluation was released, it clearly shows that the concept of restriction to few pivotal studies with focus on reproduction raises major problems.

The published prospective epidemiological studies on infants in the Netherlands support concern that the TDI proposed by the SCF may be under-protective. Dutch infants from mothers exposed to a median daily dose of 3 pg TEQ (PCDD/PCDF/dioxin-like-PCB)/kg bw per day and non dioxin-like PCBs showed effects on cognitive and motor development as there are lower psychomotor scores and lower cognitive abilities (e.g. Vreugdenhil et al. 2002, Patandin et al. 1999c).

4.7 Dioxin-like compounds as endocrine disrupters

Effects of dioxin-like substances on sexual dimorphic behaviour, brain morphology and sex ratios in offspring as discussed above and the link between

dioxin body burden and altered menstrual cycle characteristics observed in the Seveso cohort as described by Eskenazi et al. (2002b) point to dioxin-like compounds playing a role as endocrine disrupters in the system of sex steroidal hormones. The role of dioxin-like substances in disruption of the thyroid hormone system has been extensively reviewed (IPCS 2002).

Endocrine disrupters in general and dioxin in particular (Zareba et al., 2002) can exert non monotonic dose- response curves and can show different effects in different windows of critical exposure. This can be crucial for the extrapolation to low doses as routinely done in the derivation of critical doses such as TDI. Unfortunately this has not been discussed in the SCF documents though this may contribute to underestimation of the substance's potencies at low, environmentally relevant doses.

4.8 Immunological effects

For the first risk assessment (SCF, 2000) on dioxin in food, the SCF considered the study of Burleson et al. (1996) on virus host resistance. B6C3F1 mice were dosed with 1-100 ng/kg TCDD and challenged with Hong Kong influenza virus seven days later. The dose groups receiving 10, 50, and 100 ng/kg showed increased mortality while 1 and 5 ng/kg had no effect on influenza induced mortality. TCDD induced enhanced mortality to influenza virus in mice following a single dose of 10 ng/kg is still one of the most sensitive adverse effects described yet. Inclusion of these results in a risk assessment would lead to a much lower TDI than those proposed by the SCF.

Because of the “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”, SCF decided not to include this study in the set of pivotal studies used for the derivation. Additionally it was argued by the SCF that a dose-response relationship was lacking (though the effects were clearly dose-related) and that epidemiological studies in Seveso children with chloracne only showed minor transient changes in the immune system parameters (SCF, 2000).

In the light of the recent epidemiological study by Weisglas-Kuperus et al. (2000) in Dutch preschool children this decision of the SCF should be challenged. Weisglas-Kuperus et al. found that perinatal PCB exposure was associated with the change in cellular immune markers and lower antibody levels to mumps and measles. A higher dioxin TEQ was found to be associated with a higher prevalence of coughing, chest congestion, and phlegm.

These children were perinatally exposed to PCB background concentrations probably for their mothers in the vicinity of the TDI of 2 pg/kg bw per day proposed by the SCF. Though it is difficult to derive a TDI from the results of this epidemiological study, it strongly indicates that the SCF may underestimate the risk for adverse immunological changes due to exposure to dioxin-like substances.

4.9 Carcinogenicity

The SCF-re-evaluation (SCF, 2001) did not discuss the carcinogenic properties of dioxins/PCBs. In the SCF's first evaluation of November 2000 carcinogenicity was addressed under Chapter 3.3. 'Effects considered as not being critical in the derivation of the tolerable daily intake'.

Dioxins have proved to be carcinogenic and tumour-promoting in animal experiments. Particularly, experimental data show TCDD to be carcinogenic for a number of target organs in different species and both sexes even at doses below the maximum tolerable dose (MTD). Observed effects were alteration in gene expression, receptor-modulated effects, increase of cell proliferation, increase in growth rate of initiated cells, suppression of apoptosis and suppression of cellular interactions/communications (EPA, 2000).

A fairly consistent finding for exposed workers is a mortality rate higher than the normally observed "healthy worker effect", for all tumours (SMR for men for all mortalities of the "international cohort" 0.97, 95 % CI 0.94-1.00, Kogevinas et al., 1997). With the exception of sub-cohort II in Becher et al. (1996), the standard mortality rates listed in Table 1 are all between 1.13 and 1.6. Equally consistent is the increased incidence of tumours of the lymphohematopoietic tissue, with an SMR of 1.8 to 12.04 (e.g. non-Hodgkin's lymphomas, multiple myelomas and leukemias; Table 1). An increased incidence of tumours of the lymphohematopoietic tissue was also found with exposure to high environmental concentrations (Seveso). Individual studies showed increased mortality rates for respiratory tract and lung tumours (Table 1).

The epidemiological data were obtained using groups simultaneously exposed also to other substances, mainly phenoxy herbicides such as 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 2,4-dichlorophenoxy acetic acid (2,4-D) or chlorophenols. 2,4-D, 2,4,5-T and chlorophenols (excluding PCP; see, however, Ramlow et al., 1996) are not classified as carcinogenic or mutagenic. Also, assessments of 2,4,5-trichlorophenol and 2,4,5-T have produced no evidence of carcinogenicity where the substances did not contain TCDD as impurity (MAK, 1995a, b).

Moreover, the epidemiological data demonstrate a link to TCDD in several respects:

- Based on blood TCDD analyses and retrospective extrapolations to the exposure period, they show standard mortality rates to increase with the dose (Flesch-Janys et al., 1998a, b; Hooiveld et al., 1998; with qualifications: Ott and Zober, 1996).
- Flesch-Janys et al. (1998a, b) point out additionally that exposure to other carcinogens occurred mainly with low simultaneous exposure to TCDD.
- The elevated risks (Hooiveld et al., 1998; Bertazzi et al., 2001) or elevated standard mortality rates (Ott and Zober, 1996; Collins et al., 1993) found after accidents involving relatively short-term exposure can be plausibly explained by the very long half-life of TCDD (half-life of TCDD: average of 7 years, see above; 2,4,5-T: average of 23 hours, MAK, 1995a; 2,4,5-trichlorophenol: no data, rat: average of 24 hours, WHO, 1989).
- In the “international cohort” study, which also included sub-groups not exposed to TCDD, the standard mortality rate of the group exposed to TCDD was shown to be higher than that of the non-exposed group (Kogevinas et al., 1997).
- The epidemiological data show standard mortality rates to increase with the time elapsed since first exposure to TCDD (Hooiveld et al., 1998; Kogevinas et al., 1997; Bertazzi et al., 2001) or with the duration of exposure to TCDD (Steenland et al., 1999; Kogevinas et al., 1997).

Based on these data, which are supported by data from animal experiments, a causality of the elevated tumour rates for humans and exposure to TCDD can be assumed. According to the knowledge gained from animal experiments, the carcinogenic effect seems to be mediated via the Ah receptor.

It can be assumed, however, that TCDD is responsible for the elevated tumour rates observed. Therefore, Germany has classified TCDD as “substance known to be carcinogenic to man” (Carc. Cat. 1) in accordance with Annex 1 to EU Directive 67/548/EEC (EEC, 1967).

The quantitative risk estimates (using non threshold models) on the basis of epidemiological studies and animal experiments have neither been mentioned nor discussed by SCF.

As regards this point, EPA (2000) calculated its most current upper bound slope factor at approximately $1 \cdot 10^{-3}$ per pg TCDD/ kg bw per day for estimating human cancer lifetime risk based on human data.

A recent risk assessment (Gastel, 2001) tried to overcome the controversy with respect to the mechanisms of TCDD carcinogenicity (genotoxic/ non genotoxic ; threshold versus non threshold). He used early indicators of response for non-genotoxic carcinogens in its 'biologically based risk assessment'. Using CYP1A1 as the sensitive marker, an acceptable daily intake of 5-50fg TCDD/ kg bw per day was estimated. This is similar to the current EPA recommendation.

The difference between these estimations of TCDD associated risks and the SCF's proposal is obviously large and needs to be clarified. At least it may be indicative of the difficulty of setting a precise tolerable daily intake for TCDD on the basis of a generally accepted scientific procedure.

The opinion expressed in the Committee's evaluation (SCF, 2000) that the rat is more sensitive than humans are, is based on a comparison of the body burdens from the 2 year carcinogenicity study in rats by Kociba et al. (1978) and human body burdens from epidemiological studies. The corresponding body burdens in the rat from 294 ng 2,3,7,8-TCDD/ kg bw (LOAEL) to 2976 ng 2,3,7,8-TCDD/kg bw at the highest dose (100 ng/kg bw) is obviously in the same range as estimated human body burdens from epidemiological studies (109 - 7000 ng/kg bw). Therefore the conclusion of the SCF that the rat is more sensitive than humans can not be followed.

SCF assumes that a threshold model would be the appropriate model. The rationale for this assumption does not become transparent from the SCF paper. In addition it is not clear which threshold is the relevant one (cell growth and differentiation, induction of cytochrome P450, inhibition of apoptosis) and what threshold dose is to be used in a risk evaluation.

4.10 Related compounds

The question whether 'related compounds'(dioxin-like compounds) should be included in the risk assessment process for Dioxins and dioxin-like PCB was discussed briefly by the SCF in its first opinion of November 2000 (SCF, 2000).The re-evaluation from May 2001 (SCF, 2001) does not even mention this issue. As example the proposal of van Birgelen (van Birgelen, 1998) has been

used to treat hexachlorobenzene as a major contributor to the dioxin activity in human milk. The Committee concluded that the TEF-approach could not be applied on the basis of current knowledge, mainly because some of the effects of HCB differ from those induced by TCDD. The controversy whether HCB has to be included or excluded from the application of the TEF concept has been discussed elsewhere (e.g. Vos, 2000; van Birgelen, 2000).

In our opinion HCB should be a candidate for the inclusion into the TEF concept. This is because the assumptions which have been made to derive consensus TEFs and the uncertainties associated with the TEF concept (Van den Berg et al., 1998) indicate that HCB could be included because it fulfils the basic requirements. Non Ah-receptor mediated/related effects have been explicitly excluded in the derivation of the TEF concept, therefore these effects can also occur, but may not be used to exclude the application of the TEF concept. With respect to the contamination, HCB would add 10-60 % to the total TEQ in human milk (van Birgelen, 1998).

Another group of substances, the polychlorinated naphthalenes (PCNs), consisting of 75 congeners, show similar properties to PCBs. A number of them show dioxin-like toxicity. The REPs (10^{-3} - 10^{-6}) assigned to these dioxin-like congeners – most potent are the penta-, hexa- and hepta – chlorinated naphthalenes – are in the same range as the TEFs of some of the dioxin-like PCBs (Van de Plassche and Schwegler, 2002).

The concentrations of PCNs in Swedish human milk samples from 1972-1992 (Norén and Meironyté, 2000) are only near or even below 1ng/g milk lipid and therefore would not grossly contribute to the total TEQ of human milk. Whether this is also true for other countries has to be investigated. Data from fish samples show however, that PCN-TEQ can contribute to the overall TEQ as much as PCB-TEQs (Kannan et al., 2000). Thus PCNs can contribute significantly to the overall TEQ in food and should therefore not be neglected in tolerable intake considerations.

A third example of another class of ubiquitous, bioaccumulative dioxin-like compounds which bind to the Ah-receptor and induces Cyp1A1 are polybrominated diphenylethers (Bunce et al., 2001). The mean levels of 4 ng/g milk lipid are only about 1 % of the PCB levels in Europe, but in USA and Canada the average contribution can reach 10 %. Peak levels can be as high as the PCB levels (Ryan et al., 2002b).

In addition to these three examples of dioxin-like substances/substance groups a number of other dioxin-like or suspected substances (Van den Berg et al., 1998) can contribute to the overall TEQ intake of humans.

It is acknowledged that it is difficult at present to include these dioxin-like substances with precise specific TEFs into a TDI, but the above examples make it clear that any TDI based only on PCDD/F and dioxin-like PCB underestimates the real overall TEQ intake. Therefore this uncertainty has to be included in the risk assessment process.

In the case of the WHO recommendation for a TDI-value of 1-4 pg WHO-TEQ/kg bw per day (WHO, 2000) one would favour at least the lower end of the proposed range, that means 1 pg WHO-TEQ/kg bw per day.

5. Conclusions

Although it is acknowledged that any recommendation of a precise number for a TDI is flawed by uncertainties and the possibility of different weight being given to the studies of relevance, we come to the conclusion that

1. The SCF – reevaluation should not be supported because
 - the SCF does not adequately indicate uncertainties in its risk assessment and therefore does not apply adequate uncertainty factors.
 - the SCF does not assess risks for immunological, behavioural and carcinogenic effects though animal experiments and epidemiological data indicate that these effects may occur at lower doses than the developmental effects it characterized as pivotal.
 - the SCF does not discuss the epidemiological data on developmental effects and their importance to the SCF's recommendation and does not take into account that endocrine disrupting effects of dioxins which may exert non monotonic dose response curves.
 - the SCF should recalculate the EHDIs using a more reliable absorption factor and overall TEQ half life times.
 - the SCF does not take into account that any TDI based only on PCDD/F and dioxin-like PCB underestimates the real overall TEQ intake because other dioxin-like compounds are not included.
2. The increase of the TDI is a signal pointing to the wrong direction, allowing the deterioration of all regulatory limit values that are based on the TDI value.
 - the doubling of the TDI lowers the level of protection for humans though breast-fed infants in particular are exposed to a level that is highly undesirable.

- any additional exposure would prevent the achievement of intake levels below 1 pg TEQ/kg bw per day and would persist over decades because of the overall TEQ half life of about 15 years.
3. It is proposed not to follow the SCF's recommendation of a TWI of 14 pg WHO TEQ/kg bw.
 - It is proposed to use the lower end of the WHO TDI range of 1 pg/kg bw per day for all standard settings and risk reduction measures.
 - It is proposed to follow the WHO proposal to set a TDI instead of a TWI because a TWI does also not reflect adequately the long half lives of the substances under consideration. In addition a TWI makes it more complicated to evaluate intakes in total diet studies and to derive controllable TWI - related limit values in food and feed.
 4. It is proposed to maintain the goal set by the WHO “ to reduce human intake levels below 1 pg TEQ/kg bw per day”
 - because the Risk Assessment performed does not consider potential effects in breastfed children caused by the undesirable high contamination of mothers milk
 - because the LOEL/LOAEL of ‘effects which may or may not lead to adverse effects’ (WHO, 2000) and the related body burdens in experimental animals have also not been considered in the derivation of the TDI
 - because the carcinogenic risk of the background exposure may be according EPA (2000) unacceptable high for the general population.

Finally it is proposed, to reassess the TDI in a process transparent to the public on the basis of all relevant endpoints from animal experiments and human epidemiology, including the assessment of cancer risks.

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