SUMMARY REPORT

EFSA SCIENTIFIC COLLOQUIUM

Methodologies and principles for setting tolerable intake levels for dioxins, furans and dioxin-like PCBs

28-29 June 2004, Brussels, Belgium

3 September 2004
Preface

EFSA Science Colloquia aim to achieve a better understanding of the fundamental scientific issues related to risk assessment on food and feed and are therefore organised in a way to provide ample opportunity for an interactive exchange of expert views. To that end the Science Colloquia are sufficiently informal to allow for substantial debates if needed, however, at the same time, they are adequately structured and managed to enable participants to reach conclusions and make recommendations, as appropriate. The meeting on methodologies and principles for setting tolerable intake levels for dioxins, furans and dioxin-like PCBs was the first in this series of Science Colloquia.

There are differences in approaches used by authorities in assessing the risks of dioxins and dioxin-like compounds to human health including extrapolation from effects in the observable high dose range to background dietary exposure. A publication in January 2004 in the journal *Science* on the global assessment of organic contaminants in farmed salmon (Hites et al., 2004. Science 203: 226-229) triggered substantial comments from scientists and media around the world. The article was based on data concerning the occurrence of persistent organic pollutants found in a variety of farmed and wild fish. While the data collected were considered valuable, experts disagreed about the approach followed by the authors in assessing the comparative health risks associated with the consumption of farmed and wild salmon. Consequently, the article’s recommendations with respect to the consumption of safe amounts of salmon were highly controversial and potentially confusing for consumers. The European Food Safety Authority took up this issue at its first Scientific Colloquium where recognised experts in the field discussed scientific aspects and issues relating to the setting of tolerable intake levels for mixtures of polychlorinated dioxins, furans and dioxin-like PCBs. In order to allow for international discussions, participation was not limited to European experts but was extended to experts from North America and Japan, including the authors of the *Science* article.

The objectives of this first colloquium were to analyse and discuss the differences in approaches and principles for setting tolerable intake limits for mixtures of polychlorinated dioxins, furans and dioxin-like PCBs, update the state-of-the-science on the subject, and, finally, to consider the possible impact of new developments, if any, on consumer advice.

We are very pleased with the enthusiasm of all participants, the very open and lively discussions and the outcome of the meeting. Special appreciation is expressed to the Co-Chairs of the Colloquium, the Chairs and Rapporteurs of the various break-out sessions and, in particular, to Diane Benford and Dieter Schrenk who have been so kind to draft the report of the meeting.

Herman B.W.M. Koëter, Deputy Executive Director and Director of Science, Juliane Kleiner, Senior Scientist and Scientific Coordinator ad interim of the Scientific Panel on Contaminants in the Food Chain
I Introduction

The health significance of human exposure to dioxins (polychlorinated dibenzo-p-dioxins, PCDDs) furans (polychlorinated dibenzofurans, PCDFs) and polychlorinated biphenyls (PCBs) has been the subject of extensive discussions. Recent assessments of the risks of human health from dioxins, furans and dioxin-like PCBs were performed by WHO (1998), EC Scientific Committee on Food, (SCF 2000 and 2001), WHO/FAO Joint Expert Committee on Food Additives (JECFA, 2002) and by various national authorities all over the world. However there are some differences in approaches used by various authorities for the assessment of dioxins and dioxin-like compounds to human health risk including extrapolation from effects in the observable high dose range to background dietary exposure.

To analyse and discuss these differences and to update the state-of-the-science and its potential impact on setting tolerable intake levels, EFSA organised its first Scientific Colloquium on 28-29 June 2004 in Brussels, Belgium (the programme of the Colloquium is attached as Annex 1). About 60 experts representing relevant expertise in the field participated in an interactive exchange of expert views (list of participants is attached as Annex 2).

After a number of introductory presentations (copies of the presentations are attached as Annex 3), participants were split up in small discussion groups to discuss the following issues in more detail:

Discussion Group 1: Mode of action and toxicity equivalence, species differences and human variability in the Ah receptor

A Mode of action and toxic equivalence

The starting point for risk assessments of dioxins (PCDDs), furans (PCDFs) and dioxin-like PCBs in food is a risk characterisation of 2,3,7,8-TCDD (2,3,7,8-tetrachlordibenzo-p-dioxin). No matter if it has been expressed as a tolerable weekly or monthly intake or as a risk specific dose, the risk assessment is extended to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs by converting the exposure results into toxic equivalents (TEQ). This conversion is based on the assumption that all 2,3,7,8-substituted PCDDs and PCDFs, as well as the dioxin-like PCBs, have the same mode of action, elicited by binding to the same receptor, the Ah receptor, and show comparable qualitative effects, but with different potencies. These differences in toxicity are expressed in the toxic equivalency factors (TEFs), estimated from the weaker toxicity of the respective congener in relation to the most toxic congener 2,3,7,8-TCDD, which is assigned the arbitrary TEF of 1. Consensus on the TEFs for PCDDs, PCDFs and dioxin-like PCBs for both human (WHO-TEFs) and fish and wildlife risk assessment was obtained at a WHO meeting in 1997 (van den Berg et al., 1998). The criteria used by WHO for including a compound in the TEF scheme for dioxin-like compounds were that the compound must:
- show a structural relationship to the PCDDs and PCDFs,
- bind to the Ah receptor,
- elicit Ah receptor-mediated biochemical and toxic responses, and
- be persistent and accumulate in the food chain.

The following points should at least be considered:

- Are there new data that may necessitate a revision of the TEF values for PCDDs, PCDFs and dioxin-like PCB?
- Should the TEQ concept be enlarged to embrace other (persistent) compounds than PCDDs, PCDFs and dioxin-like PCBs?
- The use of the TEQ-concept assumes dose additivity. Is this assumption valid for all the congeners included by the WHO?
- Are the most sensitive end-point (related to reproductive and developmental toxicity, endocrine toxicity, immunotoxicity, carcinogenicity) all elicited through binding to the Ah-receptor?
- Do the TEF values currently assigned adequately reflect the potency differences between the congeners as regards the most sensitive end-points (related to reproductive and developmental toxicity, endocrine toxicity, immunotoxicity, carcinogenicity)? Note: Possible differences in biodisposition between congeners are discussed in DG 4.

**B Species differences and human variability in the Ah receptor**

The risk assessment will be likely to concentrate on two tissues – the liver and the foetus

- How big are the differences in the AH-receptor occurrence and activities between various animal species and humans?
- Are humans less sensitive to the Ah-receptor related effects than e.g. rats and monkeys?
- How much human variability is there in the Ah-receptor?
- Has human variability in the Ah-receptor any toxicological consequences?

**Discussion Group 2 and 3: Dose-response characterisation for critical effects and comparison of animal and human data**

Different risk assessment and evaluation approaches for dioxins, furans and dioxin-like PCBs have been used by various authorities in different parts of the world. The objectives of the DG 2 and 3 are to identify the critical effect(s) and the corresponding dose-response relationship and to identify the best approach for setting tolerable intake limits. The following points should be specifically considered:

- What are the critical effects?
- Role of biomarker(s) of effect(s) (adaptive vs. adverse reactions)
Mode(s) of action for the critical effects and its consequences for:
- relevance for the human situation (extrapolation from experimental animals to humans)
- threshold vs. non-threshold responses (high-to-low dose extrapolation)

The dose response relationship for critical effect(s)

Use of animal or human data for the assessment of tolerable intake limits, or both?

From the hazard characterisation, what are the conclusions for mixtures of dioxins, furans and PCB? What are the conclusions for risk additivity across classes of different contaminants?

What are the 3 most important gaps in the assessment leading to uncertainty?

Discussion Group 4: Metabolism, interspecies kinetics and human variability in target organ delivery

A Overall fate of TCDD in humans and animals – is the rat a suitable model for humans?

- Extent of absorption – influence of vehicle and food
- Distribution – are data in rats adequate for converting bolus dose data into steady-state concentrations?
- Pathways of elimination – faecal vs. metabolism
- Potential for accumulation – is half-life adequate to reflect accumulation in all tissues?

B Extrapolation of data on TCDD to other congeners – are possible differences in biodisposition between congeners allowed for adequately in TEFs?

C. The risk assessment will be likely to concentrate on two tissues – the liver and the foetus

- Are there species differences in steady-state distribution between liver and biomarkers of internal dose, such as concentrations in blood lipids or adipose tissue?
- Do any species differences in the relationship between dose and the measured concentration in the liver reflect differences in the concentration available to interact with Ah receptors? Do species differences in measured total tissue concentrations reflect differences in cytosolic concentrations available to the Ah receptors?
- Are the methods to convert the concentrations in the foetus of rats following a bolus dose (or short-term treatment) into steady-state long-term intakes valid? Does extrapolation to humans based on species-differences in half-life provide a scientifically valid approach or is a PBPK model essential?
D. How much human variability is there in the toxicokinetics of dioxin and other congeners? How should human variability be taken into account in risk assessment?

E. Is there any point in advising women who may become pregnant to reduce their intake of dioxins? Is the advice for TCDD also relevant for other congeners?

The outcomes of the working groups were discussed and conclusions drawn up during a final Plenary session. This report summarises the main results of the Colloquium.

Dr. Herman Koëter, European Food Safety Authority was the overall chairman and Dr. Ada Knaap (RIVM, NL) and Dr. Andreas Gies (German Federal Environmental Agency) were the overall co-chairs. Dr. Dieter Schrenk (University of Kaiserslautern, Germany) and Dr. Diane Benford (Food Standards Agency, UK) volunteered to be the overall rapporteurs. Dr. Rolaf van Leeuwen (RIVM, NL), Dr. Josef Schlatter (Swiss Federal Office of Public Health), Dr. John Christian Larsen (Danish Institute for Food and Veterinary Research) and Dr. Linda Birnbaum (US Environmental Protection Agency) offered to be discussion group chairs whereas Dr. Angelika Tritscher (WHO, Geneva), Dr. Richard Canady (US Food and Drug Administration), Dr. Mark Feeley (Health Canada) and Dr. Andrew Renwick (University of Southampton, UK) were the corresponding working group rapporteurs.
II Summary of the discussion results

1 Mode of action and toxicity equivalence

1.1 Role of the Ah receptor

The Colloquium agreed that activation of the Ah-receptor (AhR) is a necessary but not sufficient requirement for the toxicity of dioxins. Studies in AhR null mice demonstrate that most non-cancer effects of dioxins require AhR activation. However, the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has not been investigated in AhR-null mice. In tumour promotion experiments in mouse skin, however, dependence on the AhR was reported, demonstrating that AhR is not the only important factor in determining carcinogenic potency of TCDD.

The issue of thresholds was discussed. A ‘practical threshold’ depends on the study design and sensitivity of the methodology. More important is the question of whether there is an intrinsic physiological threshold in AhR occupation and response. Threshold-events appear to be among later events whereas the primary events may be non-threshold events. There is uncertainty about the effect of dose-response relationship for additional molecules of TCDD in the presence of endogenous ligand. The nature and role of the postulated endogenous ligand are unknown.

1.2 Toxicity Equivalency Factors

For the evaluation of congeners other than TCDD and of mixtures of dioxins the concept of toxicity equivalency factors (TEFs) has been established. Based on this concept a few congeners are predominant for background human exposure:

- 2,3,7,8-TCDD
- 1,2,3,7,8-PeCDD
- 1,2,3,6,7,8-HxCDD
- 2,3,4,7,8-PeCDF
- PCB 126

Current TEFs have been derived on the basis of all available data from studies where TCDD was included for comparison, focusing on toxic responses, and predominantly from in vivo data. There is a relatively good database for most of the sensitive endpoints for the congeners most relevant to human exposure, however, it has not been established that all of the endpoints observed in animals are relevant to humans. Findings from recent long-term carcinogenicity studies, when analysed, may provide insight as to the appropriateness of the TEF concept for characterizing potential cancer risks. However, it was considered that the TEF values may need to be reviewed based on additional data primarily for those endpoints considered to be the most relevant and/or sensitive.
TEFs have been designed as an 'order-of-magnitude' estimate and therefore a certain degree of imprecision is implicit. It was recommended that future efforts to refine the TEF concept should focus on a more rigorous evaluation and documentation of the uncertainties and improved transparency of the process. Review of the TEF values should firstly evaluate the uncertainty for each congener, e.g. strength of database, purity of the substance tested. Reporting should highlight gaps in the data, in order to support updating of the values as new data emerge. A probabilistic approach to derivation of TEFs was discussed.

The primary focus for refinement or expansion of TEF should be on the most relevant congeners for human risk assessment. Additional substances could be considered provided that they meet the current criteria, i.e. persistent, bind to the AhR, induce the same spectrum of AhR-mediated responses and structurally related to TCDD. The Colloquium confirmed that these criteria are still valid. The range of relative potencies for the polychlorinated biphenyls (PCBs) seems very large and requires confirmation. The issue of including further compounds, such as polybrominated dioxins and furans, should be discussed. Priority for initial exploration of these compounds should be based on exposure with subsequent prioritisation based on likelihood for toxicity based on screening analyses. In particular when testing AhR agonists with relatively low potency, the purity (particularly the absence of strong receptor agonists) has to be tested.

Since the current TEF/TEQ concept has been based on external dosage experiments, the modification of TEF values to take account of internal dose, could be considered.

*It was agreed that there is a need for re-evaluation of the TEFs, but it was beyond the scope of the Colloquium to identify specific compounds to be included.*

1.3 **Additivity**

Dose-additivity is a basic property of the TEF concept. The concept of dose-additivity assumes that the dose-response relationships are parallel for the different congeners. In view of the common mode of action via AhR binding, this assumption is considered to be reasonable. Most experimental data are in support of additivity whereas in complex mixtures including some non-dioxin like PCBs, deviations from additivity have been reported (i.e. combined effect is more or less than additive). This issue is considered further in section 2.3.4.

1.4 **Species differences and human variability in the Ah receptor**

The question of species differences in AhR needs further analysis. In particular, it was recognized that the mean affinity of the AhR to TCDD and a few other dioxins is lower in humans than in rodents. However a high variability of receptor affinity between human individuals may lead to higher receptor affinity in certain human individuals when
compared to rodents. It was noted that the AhR is widely distributed in the human fetus from an early stage.

The Colloquium agreed that there is a need to establish a scientific expert group to review the current database and look more quantitatively into the toxicological consequence of human AhR variability by addressing the following questions
- What is the variability in experimental animals?
- Where do humans fall within this range?
- What is the human variability in AhR?
- Are the current uncertainty factors justified?

*It was agreed that an expert group should be established to review human and species variability in the Ah receptor and downstream mechanisms leading to the adverse effects of dioxins.*

### 2 Critical effects and derivation of a tolerable intake level

#### 2.1 Critical effects of dioxins

The effects occurring at the lowest doses in animal studies result from *in utero* exposure, resulting in abnormalities in the development of the reproductive system (e.g. sperm count decreases, secondary sex accessory gland weight changes, urogenital tract malformations) and immune system. Neurological development may also be affected and neurobehavioural endpoints may be a future area for investigation. Non-developmental effects in laboratory animals include immune effects, endometriosis and cancer.

The majority of the studies involved acute bolus dosing, and require extrapolation for long term dietary exposure.

Biomarkers such as CYP induction and other changes in gene expression are not necessarily adverse per se but may provide an indication for possible adverse effects. Gene expression/array systems provide a sensitive measure of early changes. Enzyme induction and results from gene expression arrays could be used as markers of exposure but would only be considered as possible early indicators for potential adverse effects if they are demonstrated to be obligate precursors to an adverse effect and appropriate consideration is given to mechanisms of repair and homeostasis.

Cancer is recognized as a relevant endpoint in the epidemiological studies but it is still questioned whether it is a threshold or non-threshold effect. It is considered not possible to base a risk assessment solely on the current human data. Other reported effects in humans include immune effects, endometriosis, diabetes and cardiovascular disease.

Exposure is generally to complex mixtures of lipid-soluble (and other) contaminants; the on-going human studies (reproductive endpoints, bone/teeth effects, reanalysis of occupational cohorts) may be used as supportive/confirmation of effects in experimental animals and may contribute to a risk assessment in the future.
It was agreed that there is a need for further consideration of the possible human health effects such as immune effects, endometriosis, diabetes, bone, teeth, and cardiovascular effects.

2.2 Mode of action for the critical effects

As already noted, binding to the AhR was considered to be necessary, but not sufficient, for adverse effects. Based on current understanding, receptor binding is not likely to be a threshold-related event. Early associated biochemical effects also appear to be non-threshold events at the limit of experimental design, but these biomarkers are not adverse per se. More complex biological responses often seem to have a threshold; for example receptor binding can occur without resulting in cancer, although this could be related to non-linearity of the dose-response rather than to a threshold (an extreme case of non-linearity).

2.3 Dose response and risk characterisation for the critical effects

The Colloquium recognised that there are key differences in approach to assessment of the dose-response relationship for dioxins. Scientific judgement should determine the approach taken in extrapolation beyond the observed data. The WHO, JECFA and SCF dioxin evaluations considered all the data in order to select and describe the most sensitive endpoint and the critical studies within the most sensitive endpoint. Once the critical studies have been identified, the NOAEL or LOAEL are used as the basis for an uncertainty factor approach to derive a tolerable daily intake. In the case of dioxins, the US EPA provides risk estimations for all endpoints independently at the low end of the dose response range, in order to make risk managers more fully aware of the breadth of information available to inform and support decisions.

There is a need for all approaches to present more information on the dose-response data and make clear where there are differences between conclusions of the study authors and those of regulatory evaluations. The outcome of the risk characterisation is highly model dependent and the uncertainty in the model should be described. The rationale for the approach taken should be clearly described.

More interaction is needed between risk assessors, risk managers, and researchers to stimulate research of relevance to the risk assessment and to decision making.

2.3.1 Non-cancer dose response evaluation

A LOAEL/NOAEL approach may be necessary in some cases, e.g. if the key data did not include or allow a full dose-response assessment. However, alternative approaches, such as the benchmark dose approach, should be considered where possible. Factors to be
taken into account include the adequacy of the data for a particular modelling approach, and methods of quantifying uncertainty.

Given limitations in dose evaluation and statistical power, and the effects of confounding factors, epidemiological data are often most useful for a qualitative evaluation of effects in humans. The criteria for acceptance of use of human data for dose-response assessment need to be clearly described.

The WHO, JECFA and SCF concluded that the weight of scientific evidence is sufficient to assume that there is likely a dose-response threshold for the critical effects of dioxin-like compounds, including cancer. The reproductive/developmental effects (sperm counts, accessory sex gland weights) were considered to be the critical effects; these appear to follow a dose response relationship at lower body burdens than those causing other effects including cancer. However there is some uncertainty with respect to extrapolation from acute bolus to repeated dosing since internal doses in humans usually result from repeated exposure via diet and other sources. Furthermore, acute dosing may lead to particularly high internal doses at critical periods of development.

2.3.2 Cancer dose response evaluation

A margin of exposure analysis is one possible approach for the cancer endpoint assessment. For dioxin in particular, given the proximity of some estimates of effective occupational doses for cancer to food doses, there is a need to consider variability in response (i.e., susceptibility) in the evaluation of quantitative population risk estimates.

The outcome of the risk characterization is highly model dependent, and therefore the model uncertainty should be described.

Derivation of specific risk estimates below the observed range should be science-based but is sometimes a policy decision. This includes choice of default assumption of whether or not there is a threshold of effect or how to consider mechanistic evidence for toxic effects and biologically based dose-response models.

Modelling to derive consistent risk estimates as points of comparison (POC) in considering margins of exposure for cancer and non-cancer effects, for example bench mark dose methods. The POC should preferably be within the observed range.

*It was agreed that there is a need for research relating to the dose-response characteristics of early and late events in the critical effects of dioxins, in order to support decisions on the application of a threshold approach to risk assessment.*
2.3.3 Selection of animal or human data for assessment of tolerable intake levels

The Colloquium noted that the human data provide supporting evidence for the relevance of some of the effects of 2,3,7,8-TCDD observed in experimental animals. However, the currently available human data do not provide a sufficient basis for risk assessment, and it is therefore necessary to use animal data.

2.3.4 Mixtures analysis: Risk additivity

Three methods of assessing mixtures were discussed; dose additivity, response additivity, and “choosing the most toxic of the mixture.” Dose additivity allows for the simple addition of doses for individual chemicals within a mixture regardless of whether the doses are themselves below thresholds for action. Dose additivity assumes a common mechanism of action. The TEQ approach is an example of dose additivity. Response additivity allows for the addition of responses regardless of whether a common mechanism of action is known. Such addition may or may not allow addition of responses below thresholds. The US EPA approach to cancer assessment assumes response additivity in decisions by summing excess individual cancer risks for separate chemicals which have different mechanisms of action.

The agreed default approach is to allow for dose additivity when a similar mode of action exists. There is a need to characterise the uncertainty and underlying assumptions for dose additivity, including the shapes of the independent dose responses relationships. It is generally accepted for polychlorinated dibenzodioxins, polychlorinated dibenzofurans and 'dioxin-like' polychlorinated biphenyls that bind to the Ah receptor, and the TEQ approach provides a useful risk management tool for these compounds.

Dose additivity up to a maximum response is common for chemicals with a similar mode of action if measures of relative potency are available as in the case of dioxins. The colloquium noted that there is currently less convincing evidence in support of a response additivity approach across different classes of contaminants, with different modes of action, even when dealing with similar toxicological endpoints. Assumptions allowing or not allowing response additivity should be clearly described, including the scientific support for and the uncertainties in the assumptions.

The preferred method for adding potencies within or across similar modes of action is not clear yet. Alternatives include adding upper bound values or more detailed methods that incorporate or address uncertainty. Threshold/non-threshold or shapes of the dose-response functions, particularly the location of the threshold and maximum response for a given chemical with regard to the exposure level of interest, should be addressed within the response additivity approach.

Examples of criteria to be considered for adding response rates are
- No evidence of interaction that would indicate super- or sub-linear dose response
- Same type of response
• Same organs
• The available evidence indicates common general mode of action (for example, inducers versus promoters)

*It was agreed that there is a need for further consideration of response additivity (when non-dioxin-like chemicals are present), including the type of information required to define when the approach is valid.*

*The primary requirement is for research into the nature of dose-response relationships, also taking into account non-dioxin-like compounds in complex mixtures.*

### 2.4 Key information requirements

Key data gaps leading to uncertainty in the risk assessment were discussed. These included (not prioritised):

- **NOAEL for the critical effects** (in some key studies only LOAELs were observed). There is a need for longer duration studies with dietary exposures (e.g. a multigeneration study), including neurodevelopmental and endocrine effects (thyroid, gonadotropins, etc.), and information on relevant tissue doses for comparison with bolus dose studies.

- **Mechanistic data in support of the critical effects of dioxins**, including shape of the dose-response function at doses of interest, ranges in susceptibility and inter-species comparisons.

- **Identification of additional chemicals contributing to the TEQ body burden.**

- **Information on which genes or gene combinations are responsible for toxic reactions**
  - This information would help to link early dose responses (enzyme induction, etc.) to outcome and facilitate low-dose extrapolation and cross-congener comparisons.

- **Evaluation of response additivity of non-dioxin-like compounds** not currently considered in TEFs

- **Evaluation of roles of naturally occurring AhR-ligands** in overall responses to dioxins

- **Mode of action studies assist dose-response modeling**
  - For example, clarification of primary versus secondary effects related to initiation/promotion of cancer or induction of non-cancer effects
  - Are hormonal mechanisms related to critical effects of dioxin?
3 Toxicokinetic considerations in setting tolerable intake limits

3.1 Fate of TCDD in humans and animals

The Colloquium recognized that no important new data are available on the absorption of dioxins in humans. Distribution of dioxins in the body is governed by the high lipophilicity of these compounds. Induction of CYP1A2 can occur in humans and hepatic sequestration should be taken into account in physiologically-based pharmacokinetic (PBPK) models. However, hepatic sequestration by induced CYP1A2 results in increased concentration in liver but lower levels of CYP1A2 activity, hence induction can be present in animals without a change in measured CYP1A2 activity. CYP1A2 is not present in the fetus/neonate, but if induced in the mother then it could alter the toxicokinetics and hence the exposure to the fetus. Dioxins induce both CYP1A1 and CYP1A2 in rats, whereas CYP1A1 is not induced in human liver, which might explain why liver is a major target for the carcinogenicity of dioxins in rats, but not in humans. The rat appears to be a good model for the transplacental transfer of dioxins to the fetus indicating that free transfer from the maternal to the developing fetal organism occurs.

Estimates on the elimination half-lives of individual congeners have been improved, showing, for example, that elimination of TCDD in humans depends on the dose level/body burden, with half-lives in humans being shorter at higher intakes. Enhanced elimination may be associated with enzyme induction at very high doses. This may result in an underestimation of the original body burdens when these are back-calculated based on current levels, e.g. for studies on occupational cohorts. Data from Seveso will not require large back-extrapolation to define individual exposure as serum samples were taken soon after the incident, but available data for individuals are on exposure to TCDD not total TEQ.

In the case of 2,3,4,7,8-PentaCDF, which exhibits higher hepatic binding and is a major contributor to the total human TEQ exposure, further refinement of the TEF is warranted, particularly when assessing risk or toxicity on a body burden or tissue concentration basis rather than on an administered dose basis.

3.2 Extrapolation of data on TCDD to other congeners

The Colloquium discussed whether possible differences in biodisposition between congeners is adequately addressed in the TEF concept. The ratio of congeners in vivo at steady-state may be very different from the intake corrected for TEF. It was noted that all 'dioxin-like' PCB congeners may not show the same hepatic sequestration or binding as TCDD, with the exception of PCB 126 which behaves in a way similar to TCDD. 2,3,4,7,8-PentaCDF may be of increasing importance. Furthermore, exposure to dioxin-like compounds from combustion and industrial products decrease more rapidly than that to PCBs.
3.3 Concentration at the target organ

Dose-dependent induction and sequestration at high-doses (animals and humans) affects high to low dose extrapolation. Extrapolation from bolus to steady-state kinetics assumes linear kinetics, which may not be valid.

It is possible that there is nothing inherently different in the fate of TCDD in animals compared with humans. If a PBPK model is constructed based on a standard laboratory rat with a high dose of TCDD and then adjusted to a fat human and low-dose TCDD, the model predicts a half-life of about 7 years, which is consistent with the observations from epidemiological studies. Data to validate such models for humans at background exposure levels, including data on human tissue distribution (e.g., liver vs. fat) of TCDD and related compounds at general population exposure levels, remains to be developed.

3.4 Variability in toxicokinetics

The Colloquium was aware of apparently marked species differences in the toxicokinetics of dioxins. In addition to knowledge of different half-lives in humans vs. experimental animals, questions to be addressed in the future include variability in induction of CYP1A2 in the liver, which determines the fraction of the body burden in the liver.

It was considered that variability in dioxin kinetics between human individuals may play an important role for individual risk. Human variation in half-life of TCDD is about 6-fold. Therefore a default factor of 3.2 for human variability in TCDD kinetics was regarded as reasonable, although a half-life of 3-times the average of 7.5 years is not likely to result in a 3-fold higher body burden because of the time required to reach steady state. The high variability in body fat mass in humans may contribute to the variability in dioxin kinetics among humans exposed to background levels. If a long half-life is largely due to a high body fat composition, this would give a longer time to reach steady-state, and a greater total body burden at steady-state. But if there is no difference in the concentration in adipose tissue, there would be no increase in the concentrations in other tissues at steady-state and therefore there may not be an increase in risk. There could be greater variability in elimination half-lives of lower chlorinated congeners – but these are not important in the estimation of body burden of TEQs.

There is no indication for a sex difference in the toxicokinetics of dioxins, which cannot be explained on the basis of differences in body composition. Attempts to reduce body fat before or during pregnancy are not recommended because redistribution of dioxins from the adipose tissue to other tissues, including the fetus, may occur. There appears to be an age-related decline in rate of elimination, but it is currently unclear whether this is a reflection of change in body fat or some other factor.

From a scientific point of view, advice to pregnant women to reduce the consumption of certain types of food with higher levels of dioxins is of very limited value since no
significant influence on the actual body burden during early pregnancy is expected due to the long-half lives of most congeners. Attempts to reduce body fat before pregnancy are not recommended because redistribution of dioxins from the adipose tissue to other tissues including the fetus may occur. However some advice for girls/women may be possible, but it needs to be decades in advance of pregnancy, i.e. from weaning. Advice may be practical for an identified and avoidable source, but is more difficult for staple foods. Therefore advice would need to be considered on a regional not global basis.

3.5 Research needs

Key research needs (not prioritised) included:

- Understand liver to fat ratios in background exposed individuals in relation to variability in distribution (linked to body weight, fat etc)

- Investigate the relationship between the free concentration within tissues that can bind to the AhR and
  - Induction of CYP1A2, which would affect the dose-response relationship
  - Increase in body fat, which would affect all dose levels (does adipose tissue act as a reversible sink thereby lowering the free concentration for the same total body burden?) and would this give an increased or a decreased risk in those with high adipose levels?

- Derive TEFs based on internal dose to allow better comparisons of body burdens across species.

- Study dose-dependent induction of CYP1A2 in pregnant rats that had reached steady-state, linked to the effects in utero

- Define changes in human body burden over time in relation to
  - decreasing intake,
  - changes in body fat composition,
  - age
4  Transparency in assessment and characterisation of uncertainties

The Colloquium recognised the need to describe underlying assumptions as well as uncertainty in risk assessments. It was recognised that EFSA’s Scientific Committee is already considering this issue.

5  Summary and conclusions

The Colloquium agreed that there is general consensus on the science of dioxin toxicology, with respect to the state of knowledge and research needs. Differences in risk assessments and extrapolation from effects in the observable high dose range to background dietary exposure relate to the uncertainties in the interpretation of data. The Colloquium agreed that research should focus on further reducing these uncertainties. There is currently less convincing evidence to support adding potencies across different classes of contaminants with different mode of actions, even when dealing with similar toxicological endpoints. However there is a need for further consideration of response additivity including criteria to be defined when the approach could be valid. Further research is needed in order to support a harmonized approach to the issue of carcinogenicity of dioxins. A need was expressed for:

- identification of a point of comparison (equivalent response) for experimental animal or human data

- refined modelling of the available data in order to derive tolerable intake guidelines.

6  Recommendations

The Colloquium agreed the following needs for future research on:

- variability in events downstream of AhR binding and/or activation and identification of the events leading to critical endpoints of toxicity

- the possible human health effects, such as immune effects, endometriosis, diabetes, bone, teeth, and cardiovascular effects

- the dose-response characteristics of early and late events in the critical effects of dioxins in the dose range of interest for setting tolerable intake levels

- the nature of dose-response relationships for complex mixtures, also taking into account non-dioxin-like compounds

- tissue distribution of dioxins at steady-state exposure, and human variability in the distribution taking into account decreasing intakes and changes in body fat composition and age
In addition there is a need for:

- re-evaluation of the TEFs\(^1\)

- further consideration of response additivity, when non-dioxin-like compounds are present, including the type of information required to define when the approach is valid.

\(^1\) The colloquium was informed that WHO is coordinating the review of the current WHO-TEFs under the leadership of the WHO Collaborating Center in Utrecht, Netherlands
III Annexes

Annex 1: Programme of the EFSA Colloquium
Annex 2: List of Participants
Annex 3: Copies of the presentations