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DRAFT SCIENTIFIC OPINION

DRAFT Scientific Opinion on Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC)

EFSA Scientific Committee [1,2]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Synthetic and naturally occurring substances present in food and feed, together with their possible breakdown or reaction products, represent a large number of substances, many of which require risk assessment. EFSA's Scientific Committee was requested to evaluate the relevance and reliability of the threshold of toxicological concern (TTC) approach as a tool for providing scientific advice about possible human health risks from low level exposures, it's applicability to EFSA's work, and to advise on any additional data that might be needed to strengthen the underlying basis of the TTC approach. The Scientific Committee examined the published literature on the TTC approach, undertook its own analyses and commissioned an *in silico* investigation of the databases underpinning the TTC approach. The Scientific Committee concluded that the TTC approach is a useful screening tool for both qualitative risk assessment and priority setting that enables efficient use of available resources and potential reductions in animal testing. The Committee also concluded that the following human exposure threshold values are sufficiently robust and conservative to be used in EFSA's work; 0.15 µg/person per day for substances with a structural alert for genotoxicity, 18 µg/person per day for organophosphate and carbamate substances with anti-cholinesterase activity, 90 µg/person per day for Cramer Class III and Cramer Class II substances, and 1800 µg/person per day for Cramer Class I substances, but for application to all groups in the population, these values should be expressed in terms of body weight, i.e. 0.0025, 0.3, 1.5 and 30 µg/kg body weight per day, respectively. Use of the TTC approach for infants under the age of 6 months, with immature metabolic and excretory systems, should be considered on a case-by-case basis. The Committee defined a number of categories of substances that are not appropriate for which the TTC approach should not be used.

KEY WORDS

Threshold of toxicological concern, TTC, risk assessment, Cramer classification scheme.

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SUMMARY

Synthetic and naturally occurring substances present in food and feed, together with their possible breakdown or reaction products, represent a very large number of substances, many of which require risk assessment. The continuing improvements in analytical sensitivity are also resulting in the detection of a growing number of chemical contaminants in food and feed at low concentrations, as well as in the identification of substances on which there are few toxicological data.

In the light of the above considerations, EFSA needs to develop, validate and apply, where possible, practical risk assessment approaches that can be used as priority setting tools and as a means to enable more rapid provision of advice about the possibility of health risks. Such practical approaches should not in any way compromise the high scientific quality of EFSA's output. Accordingly, as a self task, the Scientific Committee was requested to evaluate the relevance and reliability of the threshold of toxicological concern (TTC) approach as a tool for providing scientific advice about possible human health risks from low level exposures, it's applicability to the work of EFSA's Scientific Committee and Scientific Panels, and to advise on any additional data that might be needed to strengthen the underlying basis of the TTC approach. The TTC approach is currently used by EFSA for evaluation of flavouring substances and for evaluation of pesticide metabolites in groundwater.

In this opinion, the Scientific Committee has considered a number of published analyses and conducted some analyses itself of both the data originally used to establish human exposure threshold values (TTC values) and data from additional studies that are included in EFSA's databases on pesticides and in an EU database of substances classified for reproductive toxicity. EFSA also commissioned a project from a contractor to examine the databases underpinning the TTC approach, using *in silico* chemoinformatic methods to assess the representativeness of the databases and the opportunities for refining the basis for grouping chemicals. Further analyses of oral toxicity data and TTC values have also been conducted and published by others using independent databases. The Scientific Committee's conclusions from this exploration of the TTC approach are as follows.

a. The TTC approach is a useful screening tool for both qualitative risk assessment and priority setting that enables efficient use of available resources and potential reductions in animal testing. The TTC approach is mostly applicable to substances for which the chemical structure is known but there are few or no relevant toxicity data. It would not normally be applied when there is a legislative requirement for submission of toxicity data.

b. For application of the TTC approach it is essential to have suitably conservative exposure assessments, which take account of high exposure scenarios. It requires information on known or predicted human exposures, for which there is confidence that they are not an underestimate. The EFSA Panels already have in place suitable exposure assessment methodologies for predicting or estimating average and high exposures in relevant sub-populations, and the EFSA Comprehensive European Food Consumption Database is expanding.

c. The classification of chemicals according to chemical structure is an important component of the current TTC approach. The classification scheme most widely used is that described by Cramer et al. (1978). The Scientific Committee is mindful that this scheme is based on the metabolic and toxicological information available at that time. With advances in knowledge over the last three decades, revision and refinement of the scheme would be timely. Nevertheless, the Scientific Committee's analyses, together with those of the EFSA's commissioned project and several other published studies (referenced elsewhere in this report) have demonstrated that the application of the Cramer classification scheme in the TTC approach is conservative and therefore protective of human health. In this respect, the Cramer scheme can be regarded as fit-for-purpose in the context of regulatory advice.

d. The Scientific Committee notes that the TTC value for Cramer Class II substances derived by Munro et al. in 1996 was on the basis of toxicological data on very few substances. Databases compiled subsequently have similarly found few chemicals classifiable as Cramer class II,

apart from flavouring substances. The Committee considers that the TTC value for Cramer Class II is not well supported by the presently available databases and therefore concludes that consideration should be given to treating substances that would be classified in Cramer Class II under the Cramer decision tree as if they were Cramer Class III substances.

e. The Committee's analysis of the lowest 10th percentiles of the NOELs in the database of Munro et al. (1996) for substances in Cramer Class I and Class III, and confirmation by others of similar NOELs using a different dataset (Escher and Mangelsdorf, 2009) demonstrate that the respective TTC values of 1800 and 90 µg/person per day derived by Munro et al. are sufficiently robust and conservative to be used.

f. Following the Scientific Committee's analysis of NOELs for organophosphate and carbamate substances, the TTC value of 18 µg/person per day, first proposed by Kroes et al. (2004), is considered sufficiently robust and conservative to cover the anti-cholinesterase activity of OPs and carbamates. Removing such substances from Cramer Class III (which are the most potent substances in that class) might be considered to have an impact on the existing TTC value for Cramer Class III. However, pending any future revision of the TTC approach, the Committee concludes that it would be prudent to maintain the value for Cramer Class III at 90 µg/person per day.

g. The Scientific Committee considers that addition to or subdivisions of existing Cramer Classes are likely to detract from the advantageous features of the current TTC scheme, that is, its ease of use, maintaining consistency in application of the approach, and its in-built conservatism.

h. Following the Scientific Committee's analysis of NOELs for reproductive and developmental toxicity for substances classified as such under EU legislation, the TTC values for Cramer Classes I and III are considered sufficiently protective for adverse effects on reproduction or development.

i. Substances with endocrine-related toxicity have been assessed extensively and comprehensively in hazard and risk assessment procedures that were in place when the Munro et al. (1996) database was compiled. They encompass a wide range of endocrine-mediated adverse effects including reproductive and developmental toxicity as well as, for example, thyroid and adrenal toxicity. In addition, the Scientific Committee's analysis of the more recent data on reproductive and developmental toxicity, based on studies using existing globally harmonised test protocols, showed that the TTC values are adequately protective. The analysis of the substances in the lowest 10th percentile of the Cramer Class III group in the Munro et al. database also indicated that adverse effects on reproduction and development were likely to be covered by the existing TTC value. It is concluded that adverse effects of endocrine-related toxicity are adequately covered by the existing TTC values.

j. For substances with a structural alert for genotoxicity, the TTC value of 0.15 µg/person per day was derived by Kroes et al, 2004. This is sufficiently robust and conservative to be used in EFSA's work, provided the structures already designated as high potency carcinogens are excluded from the TTC approach. The Scientific Committee is aware that further substances have been added to the CPDB since this value was derived. However, because a large number of substances was already in the CPDB, the Committee does not consider that the TTC value for substances with structural alert for genotoxicity would change appreciably.

k. The Scientific Committee has considered the possibility that a genotoxic metabolite could be produced from a parent substance without any structural alert for genotoxicity. If such metabolites were to be predicted, then the TTC value of 0.15 µg/person per day should be applied. The Scientific Committee recognises that there is no general agreement at present on how such metabolites could be predicted.

l. The original FDA Threshold of Regulation value of 1.5 µg/person per day is of historical importance, but has little practical application in the overall TTC approach. This is because substances without structural alerts for genotoxicity can proceed down the TTC decision tree to be considered in relation to the higher TTC values for Cramer Classes I and III (unless they are OPs or carbamates). Non-genotoxic carcinogens are considered to be thresholded and, in general, NOELs for these are in the same range or higher than NOELs for others non-cancer endpoints. The Cramer Class TTC values are therefore also applicable to substances for which it is not known whether they may be non-genotoxic carcinogens.

m. The Scientific Committee also notes that the work of the EFSA-commissioned project demonstrated that the range of structures in the two main datasets, which underpin the human exposure threshold values, are broadly representative of the world of chemicals, in terms of chemical space, as described by molecular descriptors encompassing both structural features and physicochemical properties. This provides further confidence in the general utility of the TTC approach.

n. A number of proposals have been put forward for adjusting TTC values for shorter than chronic durations of exposure. The Scientific Committee is not confident about the general applicability of these proposals and also notes that the current TTC values are derived from databases that do not address effects from acute exposure. Instead, such situations should be addressed case by case, for example by considering the margin between the unadjusted TTC value and the estimated dietary intake.

o. For application of the TTC approach to the whole population including infants and children, all TTC values should be converted to corresponding values that take into account body weight (see Figure 2).

p. The Scientific Committee has also considered whether the TTC approach could be applied to young infants under the age of 6 months, in whom metabolic and elimination processes are not yet mature. If the estimated exposure is in the range of the TTC value, careful consideration would need to be given as to whether the outcome of the TTC approach can be used.

q. The Scientific Committee has considered whether the TTC approach could be applied to routes of exposure other than oral. For the oral to dermal route, default procedures are available that could be used to predict systemic exposures. However, it should be borne in mind that portal of entry effects would not be covered and these may be of relevance. It would therefore be preferable to develop TTC values from a dermal toxicity database. For the inhalation route, it would also be desirable to further extend the toxicity database that has been compiled by Escher et al (2010) before recommending TTC values for inhalation.

r. The Scientific Committee considered whether routinely undertaking metabolic prediction would be helpful for application of the TTC approach other than for prediction of genotoxicity. As the Cramer decision tree and the databases used to derive the TTC values for non-cancer endpoints reflect at least in part the toxicity of metabolites formed in the test species, the Scientific Committee concluded that it is not essential to undertake such metabolic prediction. However, there may be situations where this would be helpful, e.g. in cases where metabolic data on closely structurally-related substances are available (such as in the case of flavourings).

s. The Scientific Committee considered both previously proposed exclusions and additional exclusions that might be necessary and concludes that the TTC approach should not be used for the following (categories of) substances:

- High potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds)

- Inorganic substances
- Metals
- Proteins
- Substances that are known or predicted to bioaccumulate
- Substances with structures that are not adequately represented in the original databases from which the TTC values have been derived, e.g. nanomaterials and radioactive substances
- Substances likely to have the potential for local effects on the gastro-intestinal tract

t. Areas within EFSA's remit in which the TTC approach may be useful include, but are not necessarily limited to, low-level exposures to:

- Substances in food contact materials and their impurities and breakdown/reaction products
- Plant metabolites and degradates of pesticide active substances
- Metabolites of feed additives formed in target species that are not covered by tests in laboratory species
- Technological feed additives
- Flavouring substances in feed
- Trace contaminants in food and feed, including bottled water
- Impurities and breakdown/reaction products in food additives

u. The Scientific Committee recognises that when the different EFSA panels apply the TTC approach to their respective areas, specific considerations may be needed.

v. Wider use of the TTC approach in EFSA's work would contribute to reducing unnecessary animal use in toxicity testing.

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BACKGROUND AS PROVIDED BY EFSA

Human health risk characterisation of chemicals is normally based on substance-specific hazard data and on estimations of the level of human exposure. Whereas the latter is often based on (conservative) assumptions and theoretical models, rather than quantitative measurements and observations, the former is generally based on extrapolation of quantitative hazard characterisation data derived from resource-intensive toxicity studies in animals. The unavoidable uncertainties and assumptions made during the risk assessment process are usually covered by applying conservative safety/uncertainty factors.

Synthetic and naturally occurring substances present in food and feed flavouring agents, food contact materials, food supplements, botanicals, and food and feed contaminants, together with their possible breakdown or reaction products, represent a very large number of substances, many of which still require risk assessment. Moreover, the continuing rapid improvements in analytical sensitivity is resulting in the detection of a growing number of chemical contaminants in food and feed at low concentrations as well as in the identification of an increasing number of poorly understood substances.

In the light of the above considerations, EFSA needs to develop, validate and apply, where possible, pragmatic and practical risk assessment approaches as priority setting tools and as a means to enable more rapid provision of advice about the possibility of health risks. Such practical approaches should not in any way compromise the high scientific quality of EFSA's output.

Reconsideration of the current concept of risk assessment can be done by promoting the evolution of hazard assessment (toxicology) from a predominantly observational science at the level of *in vivo* models to a predominantly predictive science (Collins et al., 2008) focused on broad inclusion of computational models and comparative decision trees, as for example:

- Investing in new approaches, based on scientific innovation and making use of new tools and instruments such as genomics (proteomics and metabolomics) and other profiling techniques, systems biology, and biological pathway perturbations (NRC, 2007). New approaches also include concepts such as 'intelligent testing and assessment strategies' (Van Leeuwen et al., 2007), 'evidence-based toxicology' (EC-JRC, 2009), and 'conceptual risk assessment frameworks' (Goldberg et al., 1997), which are all based on step-wise risk assessment procedures defining the next step based on the outcome of the previous steps.

- Pragmatic and practical risk assessment approaches aiming at providing preliminary advice about the possibility of a human health risk. Some approaches are based on comparative analyses of hazard data from structurally - or functionally - related substances, including computational prediction of toxicity (Bassan & Worth, 2008), and use of high-throughput automated screening assays. Approaches primarily based on presumed safe levels of exposure, rather than hazard data, include the tiered assessment as applied in the REACH Regulation (EC, 2007), the threshold of regulation (TOR) concept as applied by the US FDA for food contact materials (Cheeseman et al, 1999) and, the threshold of toxicological concern (TTC) concept, which can be applied using a decision-tree approach, and which is useful for substances where human exposure levels are known to be low (Kroes et al., 2004).

In accordance with its mission, EFSA aims to invest in new risk assessment approaches based on scientific innovation and novel techniques such as genomics and other profiling methods. The Scientific Committee is also addressing new risk assessment approaches in the context of animal welfare considerations.

The use of pragmatic, science-based approaches in EFSA has already begun. In the area of risk assessment of micro-organisms, the Scientific Committee adopted an opinion on the use of the

Qualified Presumption of Safety (QPS) approach for setting priorities within the risk assessment of microorganisms used in food/feed production referred to EFSA (EFSA, 2007). This practical risk assessment approach meets the need of EFSA to assess the safety of large numbers of micro-organisms deliberately added to food and feed within an acceptable time frame.

In the area of food contact materials, the former Scientific Committee on Food and subsequently EFSA have applied a tiered approach to toxicity testing requirements, based on estimates of exposure to individual substances via migration from food contact materials into food and the principle that lower levels of exposure require less toxicity data for risk assessment (SCF, 2001).

For the assessment of the more than 2800 food flavouring substances, for which the burden of assessment is shared between EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), EFSA applies, where possible and feasible, the concept of Threshold of Toxicological Concern (TTC). This concept refers to the establishment of a generic human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health (Barlow, 2005). Therefore the safety assessment of food flavourings based on very low levels of exposure becomes possible even in the absence of substance-specific hazard data.

The TTC approach is currently not applied in EFSA in areas of risk assessment other than food flavourings and for exposure to metabolite pesticides in groundwater. It is recognised that a critical element in applying the TTC approach is the need for reliable exposure data and that estimates of exposure need to be as complete and accurate as possible, or include adequate conservatism to account for possible underestimation of exposure.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Scientific Committee is requested to prepare a scientific opinion in which it explores options for the use by EFSA's Scientific Committee and Scientific Panels and other expert groups of the threshold of toxicological concern (TTC) approach as a formalised approach for providing scientific advice about possible human health risks.

In particular the Scientific Committee is requested to:

- Evaluate the relevance and reliability of the TTC concept for application in the food and feed area, taking into account: (i) the discriminative power of the currently available databases that underpin the concept and which have been used to define human exposure thresholds, (ii) the range and number of chemical entities represented in such databases, (iii) the routes of exposure to these chemicals, (iv) the range of reported effects following exposure, and (v) the possibilities to assess – with sufficient certainty – human exposure levels through food and feed of chemical entities for which EFSA has risk assessment responsibility;

- Advise on the application of the TTC concept in areas of chemical risk assessment addressed by EFSA other than food flavourings and define the general and specific criteria for its application as a tool to provide scientific advice on the safety/risk in these areas;

- Advise on any additional data development and/or collection needed to strengthen the underlying basis of the TTC concept and its use as a practical tool for providing scientific advice about possible human health risks related to chemical exposures via food and feed.

In developing its scientific opinion the Scientific Committee is requested to take into account the experience gained by the EFSA in applying the TTC concept in the assessment of food flavouring substances, the work currently carried out by the three non-food Scientific Committees of the Commission (SCCP, SCHER and SCENIHR) (EC, 2008), and the experience gained by other

agencies and international organisations/associations including: EMA (formerly EMEA), US FDA, JECFA, WHO/IPCS, ILSI (ILSI, 2000; Kroes et al., 2005) and COLIPA.

ASSESSMENT

1. Introduction

The threshold of toxicological concern (TTC) approach is a screening tool that has been developed in order to assess substances of unknown toxicity present at low levels in the diet. Application of the TTC approach requires only knowledge of the chemical structure of the substance concerned and information on human exposure, for which there is confidence that it is not an underestimate. It utilises generic human exposure threshold values (also called TTC values) that have been established for substances grouped according to their chemical structure and likelihood of toxicity. The human exposure threshold values have been developed based on data from extensive toxicological testing in animals. There is a range of threshold values that cover cancer and non-cancer endpoints, and these can be used for substances both with and without a structural alert for genotoxicity.

At exposures below the generic human exposure threshold values, the probability of adverse effects on human health is considered to be very low. Comparison of the known or estimated human exposure to a substance with the relevant TTC value allows an initial assessment on whether or not a substance requires a more detailed assessment. In this respect, the TTC approach has the potential to be used both for qualitative risk assessment and for priority setting, to enable efficient use of available resources. Its wider use would reduce the use of animals in toxicity testing.

The TTC approach is currently used by EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for evaluation of flavouring substances in food, and for evaluation of pesticide metabolites in groundwater in the EU (SCP, 2000). Although the TTC concept was originally developed for application to substances that may be ingested by humans from the diet, its use has since been agreed in some other contexts. These include oral exposure in the following areas: genotoxic impurities in human pharmaceuticals (Müller et al., 2006; EMEA, 2006; Humfrey, 2007; FDA, 2008), genotoxic constituents in herbal substances and preparations (EMEA, 2007), and micro-pollutants and impurities in drinking water (Rodriguez et al., 2007a,b; Fawell, 2008; Australian Guidelines, 2008; Gross et al., 2010). Its use has also been proposed for assessment of consumer products (Blackburn et al., 2005), pesticide metabolites, degradation and reaction products (CRD, 2010; Melching-Kollmuß et al., 2010; Dekant et al., 2010), and industrial chemicals assessed under REACH (ECHA, 2008; Bernauer et al., 2008). Adaptation of the TTC concept is also being considered with respect to other routes of human exposure such as inhalation (Drew and Frangos, 2007; Carthew et al., 2009; Escher et al., 2010) and dermal exposure (Safford, 2008). Similar principles to those underlying the TTC approach are also being considered for use in screening of chemicals for effects on environmental species (De Wolf et al., 2005).

In this opinion, the science underpinning the TTC approach is critically examined and recommendations are made concerning the possible wider use of the TTC approach in EFSA's work. This opinion covers only the application of TTC approach to human exposures; it excludes the applicability of the TTC approach to target animal species. It also does not consider ecotoxicological risk assessment as that is not within the terms of reference for the opinion.

2. Development of the TTC concept

2.1. Underlying principles

The TTC concept has its origin in one of the fundamental principles of toxicology, that toxicity is a function of dose. When comprehensive, substance-specific toxicity data are available, they usually allow risk assessors to identify a dose or exposure, below which no adverse effects of the substance

can be detected. For substances on which there are no such data, for example in the case of degradation product, the TTC approach can be used as a screening tool.

The TTC approach could be applied *a priori* to any substance. It would not usually be used to assess substances in food for which appropriate toxicity data already exist or for which regulatory authorities normally require toxicity data to be submitted. However, it could also be useful for prioritisation of substances for further risk assessment, e.g in cases of limited toxicological data.

2.2. Derivation of human exposure threshold values for the endpoint of cancer

To cover the endpoint of cancer, a human exposure threshold value was derived by the US Food and Drug Administration (FDA) (Rulis, 1986, 1989, 1992) to be applied to substances that do not contain a structural alert for genotoxicity/carcinogenicity. The threshold value was derived by mathematical modelling of risks from animal bioassay data on over 500 known genotoxic and non-genotoxic carcinogens, based on their carcinogenic potency. Carcinogenic potencies were expressed as TD_{50}s[3] and "virtually safe doses" (VSDs)[4] were derived from these by linear extrapolation, assuming that the risks in animals are representative of those in humans. From the distribution of VSDs, a concentration of 0.5 µg/kg of diet (0.5 ppb) was derived as the value to use for the Threshold of Regulation (TOR). This can also be expressed as 1.5 µg/person per day, assuming that 3 kg of food and beverages per person are consumed daily. If dietary exposure to an individual substance was below the threshold, the FDA considered that consumers would be protected "*with reasonable certainty of no harm*", even if that substance was later shown to be a carcinogen. In 1995, the FDA incorporated this threshold value in its TOR policy for substances present in food contact materials (FDA, 1995). Under the TOR, substances used in food contact materials that are present in the diet at concentrations below 0.5 µg/kg are exempted from regulation (see appendix A for further details).

Later, Kroes et al. (2004) refined the threshold for the endpoint of cancer by deriving a lower value for substances containing a structural alert for potential genotoxicity. The same modelling approach was used as by the FDA. They first focused on identifying high potency carcinogens that would give the highest calculated risks if present at very low concentrations in the diet and after excluding them (aflatoxin-like, azoxy-, and *N*-nitroso- compounds), they derived a human exposure threshold value of 0.15 µg/person per day for substances with a structural alert for genotoxicity.

The human exposure threshold values for the endpoint of cancer are summarised below in Table 1.

Table 1: Human exposure threshold values from cancer data

Structures	Human exposure threshold value (µg/person/day)	Reference
Without a structural alert for genotoxicity	1.5	**FDA, 1995**
With a structural alert for genotoxicity	0.15	**Kroes et al., 2004**

The original FDA Threshold of Regulation value of 1.5 µg/person per day is of historical importance, but has little practical application in the overall TTC approach. This is because substances without

[3] The TD_{50} is defined as the daily dose-rate in mg/kg body weight per day for life to induce tumors in half of the test animals that would have remained tumor-free at zero dose.
[4] The VSD is an estimate of the dietary exposure to a carcinogen which could give rise to less than a one in a million lifetime risk of cancer

structural alerts for genotoxicity can proceed down a TTC decision tree to be considered in relation to the higher TTC values as discussed below.

2.3. Derivation of human exposure threshold values for non-cancer endpoints

Around the same time as the FDA was developing the TOR policy, Munro and colleagues were developing the TTC concept (Munro 1990, 1996; Munro et al., 1996, 1998, 1999). They proposed the use of generic thresholds for acceptable human exposures based on an exploration of the relationship between chemical structures and toxicity (Munro et al., 1996). They compiled a large reference database (in this document referred to as the Munro et al. database) consisting of 613 chemicals for which oral toxicity data were available on a variety of non-cancer endpoints from sub-chronic, chronic, reproductive and developmental toxicity studies. Over 2900 no-observed-effect levels (NOELs[5]) were available from these studies.

The chemicals in the Munro et al. database were divided into three structural classes, based on a "decision tree" developed earlier by Cramer et al. (1978). Cramer Class I were chemicals of simple structure, with efficient modes of metabolism, suggesting low oral toxicity; Cramer Class III were chemicals with structures suggesting significant toxicity or which did not permit any strong initial presumption of safety, and Cramer Class II were chemicals with structures that were less innocuous than Cramer Class I but without features suggesting significant toxicity (see section 3.1 for further details). Human exposure threshold values were derived by taking the lower 5th percentile value of the distribution of NOELs for the substances in each of the three Cramer structural classes, multiplying by 60 to convert the values expressed as mg/kg bw per day into mg/person per day, and then dividing by a factor of 100 to ensure a margin of safety. The three human exposure threshold values derived for non-cancer endpoints are summarised below in Table 2.

Table 2: Human exposure threshold values from toxicity data from Munro et al, 1996

Cramer Structural Class	Fifth percentile NOEL (mg/kg bw per day)	Human exposure threshold (µg/person per day)
I	3.0	1800
II	0.91	540
III	0.15	90

More detailed information on the development of the TTC concept and the derivation of the human exposure threshold values is given in Appendix A.

2.4. The TTC decision tree

Many of the above recommendations were incorporated into a decision tree by Kroes et al, (2004) shown in Figure 1 below.

Subsequently, Felter et al. (2009) have suggested further refinements to the TTC decision tree. One of their proposals allows for consideration of any available genotoxicity data on substances that have structural alerts for genotoxicity (Step 2 of the decision tree). If the genotoxicity data are negative (e.g. Ames test and/or other data), they proposed using a higher threshold value of 1.5 µg/person per day, rather than the value of 0.15 µg/person per day recommended at Step 4 of the decision tree. The other issue they addressed was duration of exposure. The existing human exposure threshold values assume a lifetime of exposure. Felter et al. proposed using a higher threshold value of 1.5 µg/person per day in cases where dietary exposure to a chemical with a structural alert for potential genotoxicity is less than 12 months (see section 4.10.2 for further discussion).

[5] NOEL was the term used by Munro et al. Nowadays, it would be more usual to make a distinction between NOELs and no-observed-adverse-effect levels (NOAELs). NOELs are sometimes more conservative than NOAELs.

In addition to recommendations to exclude substances with structural alerts for high potency carcinogenicity (see 2.2.), Kroes et al. (2004) made a number of other recommendations for exclusion of particular groups from the TTC approach. They recommended exclusion of polyhalogenated-dibenzodioxins, -dibenzofurans and –biphenyls, which are potent substances with extremely long half-lives that show very large species differences in bioaccumulation, along with heavy metals, because they are known to accumulate in the body. Other non-essential metals in elemental, ionic or organic forms were also recommended to be excluded because they were not included in the original database of Munro et al. (1996), nor are inorganic substances covered by the structural classification scheme of Cramer et al. (1978). Proteins were also recommended to be excluded since they were not included in the Munro et al. (1996) database, and their potential for allergenicity and the potent biological activities of some peptides make them unsuitable for the TTC approach (see section 4.4 for further details).

553 **Figure1: TTC Decision Tree (Kroes et al., 2004)**
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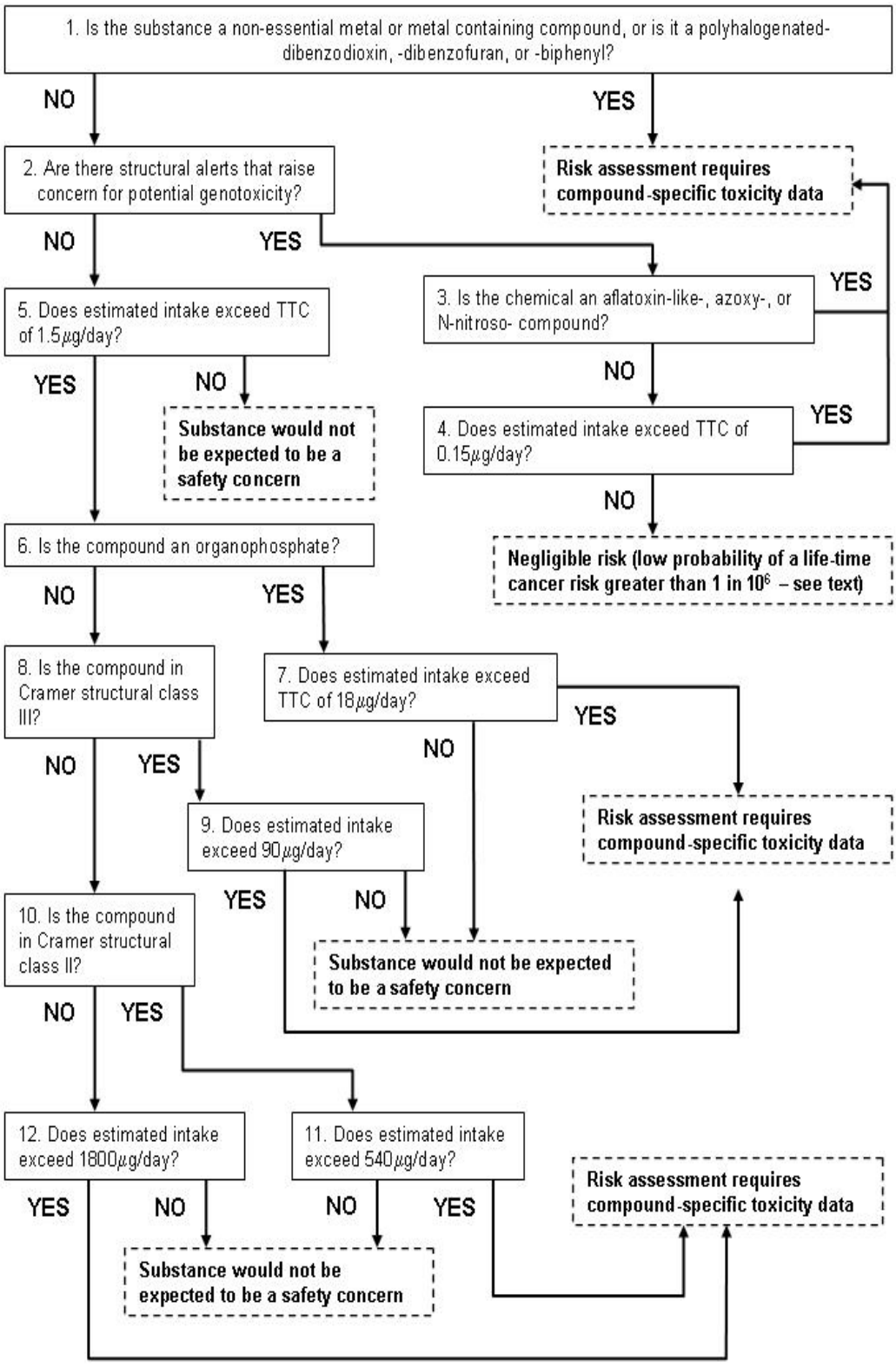

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2.5. Initial use of the TTC approach

The initial use of the TTC approach in the food area was for the evaluation of flavouring substances. JECFA was the first to consider using elements of the TTC approach for this purpose (Munro, 1996), and has since used it to evaluate about two thousand flavouring substances. These substances are usually considered in structurally-related groups, which also allows read-across in cases where there are toxicity data on one or more members of the group. The main modification made by JECFA to the generic TTC approach when applied to flavouring substances was to consider metabolism more explicitly, specifically whether a flavouring substance can be predicted by expert judgement to be metabolised to innocuous products. The modified approach was adopted as the JECFA procedure in 1996 (WHO, 1997). A similar procedure was later adopted by the European Commission's Scientific Committee on Food (SCF, 1999) and has been used by EFSA since 2004 for the evaluation of about two thousand substances on the European Union Register of Flavouring Substances (EC, 2002 and its subsequent amendments). Further information on the JECFA and EFSA procedures for evaluation of flavouring substances is given in Appendix B.

3. The Cramer classification scheme and its software implementation

3.1. Development of the Cramer classification scheme

The application of the TTC concept as described above utilises the so-called Cramer decision tree proposed by Cramer, Ford and Hall (Cramer et al., 1978) as a priority setting tool and as a means of making expert judgements in food chemical safety assessment more transparent and reproducible. They drew upon their experience in classifying food flavouring substances (Oser & Hall, 1977) and in evaluating pesticides and industrial chemicals. The criteria they proposed for the three structural classes are shown below.

Structural classes for chemicals in the TTC approach

Class I	Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.
Class II	Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.
Class III	Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

Cramer et al. (1978) based their decision tree on a series of 33 questions relating mostly to chemical structure, but natural occurrence in food and in the body were also taken into consideration. The set of 33 questions were intended as a compromise between discrimination (into the three classes) and complexity (of the questions and their ordering). The logic of the sequential questions was based on the then available knowledge on toxicity and on how chemical structures are metabolised in mammalian metabolic pathways. Some examples of the way in which substances are classified by the Cramer decision tree are as follows:

- Class I: normal constituents of the body, excluding hormones; simply-branched, acyclic aliphatic hydrocarbons; common carbohydrates; common terpenes; substances that are sulphonate or sulphamate salts, without any free primary amines.
- Class II: common components of food; substances containing no functional groups other than alcohol, aldehyde, side-chain ketone, acid, ester, or sodium, potassium or calcium sulphonate

or sulphamate, or acyclic acetal or ketal and it is either a monocycloalkanone or a bicyclic compound with or without a ring ketone.

- Class III: structures that contain elements other than carbon, hydrogen, oxygen, nitrogen or divalent sulphur; certain benzene derivatives; certain heterocyclic substances; aliphatic substances containing more than three types of functional groups.

Cramer et al. (1978) predicted that the majority of substances would fall into either Class I or Class III, rather than Class II, and that is indeed borne out by the Munro et al. database and by subsequent experience with the TTC approach. Cramer et al. (1978) tested the validity of their decision tree by classifying 81 chemicals (used as food additives, drugs, industrial chemicals or pesticides), on which toxicity data from short-term or chronic studies were available, into the three structural classes and by tabulating the NOELs. There was overlap in the range of magnitudes of the NOELs between the three structural classes, but it was clear that the NOELs of Class I substances were generally higher than those of Class III, with those of Class II being in between.

3.2. Computer-based implementation of TTC-relevant decision trees

While the Cramer classification scheme undoubtedly served to improve consistency between the toxicological evaluations made by different experts, its paper-based application requires a working knowledge of organic chemistry, biochemistry, and food chemistry, and inevitably involves a degree of subjectivity. Therefore, following a recommendation made in a JRC-ECB workshop (Saliner et al, 2005), the JRC commissioned the development of a software tool, Toxtree, to facilitate the consistent application of the Cramer scheme. Toxtree is freely downloadable from the JRC website (http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE) and from Sourceforge: (https://sourceforge.net/projects/toxtree/). In principle, Toxtree can be applied to organic molecules, organic salts, organometallic substances, and structurally well-defined oligomers and polymers. However, organometallics, oligomers and polymers were recommended for exclusion from the TTC approach by Kroes et al, (2004). The performance of the Toxtree Cramer rulebase has been evaluated by Patlewicz et al. (2008).

The current version of Toxtree (v2.1.0, June 2010), includes three rulebases relevant to TTC assessment: these are (a) the original Cramer rulebase, (b) the Cramer rulebase with extensions, and (c) the TTC decision tree of Kroes et al (2004). The Extended Cramer rulebase works by assigning substances to Class I, II, or III, according to the Cramer rules, and five extra ones described below. Some of these extra rules were introduced because it was noted that several substances were classified by Munro et al. (1996) into Class I or Class II according to the Cramer rules, even though Munro et al. reported low NOEL values upon oral administration (indicating relatively high toxicity). To overcome such misclassifications, rules (documented in the user manual, http://ecb.jrc.ec.europa.eu/DOCUMENTS/QSAR/Toxtree_Cramer_extensions.pdf) were introduced to capture the possible toxicity of these substances.

Two of the extended rules make the Cramer scheme less conservative: firstly, the list of normal body constituents is extended from 67 to over 400 so these substances are thus placed into Class I; secondly, an additional rule allows some natural phosphates to avoid automatic classification into Class III. Conversely, three additional rules make the Cramer scheme even more conservative by placing some benzene-like compounds, non-natural divalent suphur compounds, and α,β-unsaturated heteroatom compounds into Class III. On the basis of a survey carried out by EFSA and the JRC, the Extended Cramer rulebase does not appear to be widely used (see chapter 3.3).

Use of the Kroes et al. (2004) TTC decision tree results in three possible outcomes: (a) substance would not be expected to be a safety concern, (b) negligible risk (low probability of a life-time cancer risk greater than 1 in 10^6), and (c) risk assessment requires compound-specific data. Toxtree incorporates the Benigni/Bossa rules for the identification of some genotoxic carcinogens (Benigni et al., 2008), and requires the user to input the estimated daily intake.

It should be noted that the computer-based implementation of the Cramer scheme in Toxtree and other software tools (e.g. the OECD QSAR Toolbox (OECD, 2010)) has inevitably involved some decisions by the programmer, such as the chemically-based interpretation of the original rules, and the establishment of pre-defined "look-up lists" of normal body constituents and common food components.

3.3 Survey on the use of Toxtree software

In the survey carried out by the JRC, feedback was obtained from Toxtree users of the Cramer scheme, with a view to (a) identifying rules for which clarification was needed, (b) obtaining recommendations to revise, remove or add a given rule, and (c) identifying software problems or inconsistencies in the Toxtree implementation of the Cramer rulebase.

The main observations emerging from the JRC survey concerning the scientific refinement of the Cramer scheme can be summarised as follows:

i. Many of the original Cramer rules are written in a confusing and inter-dependent way, which leads to difficulties in the rationalisation of the predictions they make. These rules could be rewritten in a clearer way, possibly with modification and re-ordering.

ii. Two rules are not based on chemical features, but simply make reference to look-up lists of chemicals (Q1, normal body constituents; Q22, common food components). These could be easily extended, for example recently authorised food additives could be added to the list of common food components. Any extended lists could be peer-reviewed. Alternatively, the Cramer scheme could be recast by removing these two questions. In other words, the revised Cramer scheme would not make reference to any look-up lists (i.e. chemicals considered to be safe or otherwise), so any reference to such lists would have to be carried out separately.

iii. Some rules make references to chemical features (e.g. steric hindrance) which would need to be better explained or possibly deleted.

The Scientific Committee considers that the potential limitations of the Cramer scheme are that (a) it is based on the knowledge of the late 1970s, (b) Cramer Class II is less well defined and is sparsely populated (see also section 4.2.3.3), and (c) some structurally determined endpoints (e.g. substances with anti-cholinesterase activity) require specific consideration (see section 4.3.2).
The Scientific Committee also notes that that other additions to or subdivisions of existing Cramer Classes are being considered elsewhere. The Scientific Committee considers that if there were numerous modifications of the existing Cramer classification scheme, they are likely to detract from the advantageous features of its use in the TTC approach, that is, its ease of use, maintaining consistency in application of the approach, and its in-built conservatism.

These aspects are discussed in more detail later in the opinion.

4. EFSA's consideration of the human exposure threshold values

In evaluating the relevance and reliability of the TTC concept for application in the food and feed area, the Scientific Committee considered the question of whether the human exposure threshold values, derived by the FDA (1995) and Kroes et al. (2004) for the endpoint of cancer and by Munro et al. (1996) for non-cancer endpoints, are sufficiently conservative to apply. This requires consideration of the range of structures and number of chemical entities represented in the databases that underpin the TTC approach, whether these are sufficiently representative of the 'world of chemicals', the appropriateness of their routes of exposure, the range of reported effects following exposure, and the reliability of the NOELs and (for carcinogens) the estimates of cancer risk. These issues are discussed in subsequent sections of Chapter 4.

4.1. TTC values for potential (genotoxic) carcinogens

The TTC values covering the endpoint of cancer of 0.15 and 1.5 µg/person per day for substances with and without a structural alert for genotoxicity, respectively, are both derived from the extensive Carcinogenic Potency Database (CPDB) of Gold and co-workers (Gold et al., 1984, 1989; Gold and Zeiger, 1997) (see appendix A for details). The issue of substances with a structural alert for genotoxicity requires some further discussion in the context of possible wider application of the TTC approach in EFSA's work. As explained earlier, these threshold values were derived by linear extrapolation from the TD_{50} values obtained from animal cancer studies. However, there is no international consensus on the use of linear extrapolation from cancer bioassays to predict risks in humans.

Several approaches are currently used by risk assessment bodies and regulatory agencies in various parts of the world to assess the risks from substances with genotoxic and carcinogenic properties. For carcinogenicity, since in almost all cases adequate human epidemiological data are not available, data from animal bioassays are used, requiring extrapolation to the generally much lower levels to which humans are exposed. For extrapolation and quantitative risk assessment, several mathematical models can be used. Such models are usually based on the assumption that at low doses a linear relationship exists between the exposure level and the response for the particular endpoint. The extrapolation of data to human exposures far below the observable dose-range in experimental animals has resulted in differing predictions about human risks for the same substance, depending on the model chosen. Moreover, for any particular substance, it is not known whether or not the model chosen actually reflects the underlying biological processes.

Thus the Scientific Committee has expressed serious reservations about extrapolating from data on animal tumours observed at high doses using mathematical modelling in order to estimate risks to humans at low exposures from substances that are both genotoxic and carcinogenic (EFSA, 2005a). The Scientific Committee has recommended using a different approach for providing advice to risk managers, known as the margin of exposure (MOE) approach[6] (EFSA, 2005a). This pragmatic approach uses both intake and cancer potency data, does not require extrapolation outside the observable range in animal bioassays and it can be used for priority setting (a small MOE represents a higher risk than a larger MOE). Although the Scientific Committee acknowledged that the magnitude of an MOE which is acceptable is a societal judgment and is the responsibility of risk managers, the Committee proposed that in general a MOE of 10,000 or higher, if it is based on the $BMDL_{10}$ [7] from an animal study, would be of low concern from a public health point of view. However, the MOE approach does not generate a numerical upper bound risk estimate that could be used in deriving a TTC.

However, the Scientific Committee has also stated (EFSA, 2005a) that as the high doses applied in carcinogenicity bioassays usually elicit significant toxicity with regenerative cell proliferation in target organs, linear extrapolation from experimental data to estimate effects at low doses may lead to a considerable overestimation of true incidence. Furthermore, the presence of homeostatic and cytoprotective mechanisms, and the abundance of cellular targets, mean that a minimum degree of interaction of the substance with the critical sites or their occupancy must be reached in order to elicit a toxicologically relevant effect. Below this critical (threshold) level of interaction, homeostatic mechanisms would be able to counteract any perturbation produced by xenobiotic exposure, and no

[6] The margin of exposure is defined as the reference point on the dose-response curve (usually based on animal experiments in the absence of human data) divided by the estimated intake by humans.

[7] The $BMDL_{10}$ (benchmark dose lower confidence limit 10%), represents the lower bound of a 95% confidence interval on a BMD (benchmark dose) corresponding to a 10% tumor incidence above the control incidence. The choice by the SC of a 10% incidence (rather than 5%) as the benchmark response (BMR) was based on the fact that in most cases a tumor incidence of 10% would be the lowest observable value in experimental animal studies.

structural or functional changes would be observed. The Scientific Committee concluded in 2005 that based on the current understanding of cancer biology there are levels of exposure to substances which are both genotoxic and carcinogenic below which cancer incidence is not increased (biological thresholds in dose-response).

Turning to the details of the CPDB database, it is important to note that it contains data on the most potent carcinogens known, which have been prioritised for carcinogenicity testing, for example on the basis of their genotoxicity. In the context of the TOR (see 2.2) and the TTC approach, it was noted at an early stage that some potent carcinogens have VSDs derived from the CPDB that are lower than the TOR of 1.5 µg/person per day (Munro, 1990; Cheeseman et al., 1999). Kroes et al. (2004) later identified that, for many of these substances, the VSDs were also below 0.15 µg/person per day and that a number of them fell within certain structural groups. The structural features of those groups containing the highest proportion of substances with VSDs below 0.15 µg/person per day were identified as aflatoxin-like, azoxy, and N-nitroso moieties. Accordingly, Kroes et al. (2004) proposed that these structural groups of high potency genotoxic carcinogens should be excluded from the TTC approach when applying the TTC value of 0.15 µg/person per day.

The EFSA Scientific Committee is also aware that the Scientific Committees (SCCS, SCHER and SCENHIR) of the European Commission's Health & Consumers Directorate-General are also developing an opinion on the TTC approach (EC, 2008). They have commented (personal communication) that the CPDB contains a further 15 substances for which the VSD is below 0.15 µg/person per day, and which do not fall within the three groups of high potency carcinogens recommended for exclusion by Kroes et al. (2004). In their view, this indicates that further work is necessary to strengthen the scientific basis for the TTC value of 0.15 µg/person per day for genotoxic carcinogens.

An investigation to assess the degree of conservatism in the TTC values was undertaken in a workshop in connection with the development of the TOR (Munro, 1990). A sub-set of the data in the CPDB at that time was used to estimate the conservatism of various hypothetical thresholds, making assumptions about the percentage of all chemicals presumed to be carcinogenic. For example, assuming that 10% of all substances are carcinogens (see Fung et al., 1995), the probability of any untested chemical being a carcinogen with a VSD below 1.5 µg/person per day was 4%; the corresponding percentage for the lower value of 0.15 µg/person per day was 1%. These estimates also make a worst-case assumption that any untested substance that was a carcinogen would have a potency as great as that of the 15% most potent carcinogens in the CPDB, which is unlikely. Thus, the Scientific Committee notes that while it is possible that an untested substance may have a VSD below 0.15 µg/person per day, such an outcome would have a very low probability. The Scientific Committee also notes that TTC values , based on linear extrapolation, that give a high probability of protection against carcinogenic effects would also be more than adequate to protect against toxic effects other than cancer.

Taking all the above considerations into account, it is evident that there is conservatism in the TOR of 1.5 µg/person per day for substances without a structural alert for potential genotoxicity and in the TTC value of 0.15 µg/person per day proposed by Kroes et al. (2004) for substances with a structural alert for potential genotoxicity. The Scientific Committee therefore considers that there is a very low probability of any appreciable cancer risk to human health from exposures to untested substances below the TTC value of 0.15 µg/person per day.

Since genetic alterations include not only the possibility of cancer in somatic cells but also other effects, such as inherited changes that can be transmitted via germ cells, the Scientific Committee has also considered whether the TTC value of 0.15 µg/day would be adequate to protect against possible heritable effects from substances that are genotoxic. Based on the limited available quantitative data on chemically-induced transmissible effects, the mutation frequencies that would be associated with a TTC value of 0.15 µg/day can be calculated by linear extrapolation. Data show in all cases an

extremely low, or negligible, incremental risk, indicating that the TTC value of 0.15 µg/day is likely to cover heritable effects as well as cancer (see Appendix F for details).

4.2. TTC values for non-cancer endpoints

In order to investigate the robustness of the database compiled by Munro et al. (1996), which comprises toxicological data on 613 substances, covering endpoints other than carcinogenicity, an analysis was undertaken of aspects of the database as indicated below.

i. A review of the information in the toxicological data sources used and the criteria for data inclusion.

ii. A summary of the types of endpoints that determined the NOELs.

iii. An assessment of the original published papers and reports referenced in the database on the substances in the lowest 10th percentile of the distribution of NOELs for Cramer Class I and Cramer Class III, in order to assess the quality of the studies and whether the NOELs identified were appropriate.

4.2.1. Appraisal of sources of toxicity data used for derivation of TTC values

The reference database compiled by Munro et al. (1996) included data on chronic, sub-chronic, reproductive and developmental toxicity studies. They were mainly derived from the reports of the US National Toxicology Program (NTP), the toxicological monographs of JECFA, the Integrated Risk Information System (IRIS) of the US Environmental Protection Agency (EPA), and the Developmental and Reproductive Toxicology (DART) database compiled by the US National Library of Medicine. These sources were considered to contain well-validated toxicological data for well-defined chemical structures, covering pesticides, food additives, industrial and other types of chemical. Only studies using the oral route of administration (gavage, diet, drinking water or capsule) were included.

The majority of studies in the reference database were conducted in rodents or rabbits. Studies in other species, such as dogs, humans and ferrets, were initially included in the reference database but were not included in the final published database because they did not meet the criteria for inclusion (e.g. duration of the study was too short). In particular, dog studies were not included in the final published database due to small numbers of animals and the frequency of effects such as reduced body weight attributable to problems such as palatability and vomiting.

A further criterion for inclusion in the reference database was stated to be that studies should demonstrate a LOEL as well as a NOEL in order to ensure that a study was rigorous enough to detect toxic effects. However, a number of major food ingredients were also included in the database and these did not necessarily show toxicity, even at the highest doses tested. For such substances, which comprise 10% of the database, the highest dose tested was chosen as the NOEL in order to maintain a conservative approach.

In all, the reference database contained 2941 NOELs from studies conducted on the 613 substances, and from these the most conservative (lowest) NOEL for each substance was entered on the published database. The NOELs in the reference database were those selected by the original author(s) of each study, apart from the studies in the IRIS database, for which the NOELs selected by the EPA were used. Munro et al. (1996) commented that some authors were highly conservative in their selection of a NOEL, but such NOELs were still used for the database to maintain a conservative approach. Munro et al. (1996) also stated that in the calculation of the TTC values they divided NOELs from sub-chronic studies by a factor of 3 to approximate the NOELs that are likely to be derived from a chronic study. It was noted that the NOEL values in the Appendix provided in Munro et al. (1996) had not yet been adjusted in this way.

4.2.2. Endpoints determining the NOELs

The information contained in the Appendix to Munro et al. (1996) on the 613 substances in the published database was examined to ascertain the type of toxicological endpoint on which the overall NOEL for each substance was based, according to the study authors. The results are summarised in Table 3. Among the 613 overall NOELs, multiple effects (28 %) were reported as the most frequent endpoint, followed by body weight changes (18 %) and organ weight changes (9 %). Reproductive, hepatic and renal effects were the next most frequent endpoints.

Table 3: Reported toxicological endpoints for the NOELs for the 613 substances in the database of Munro et al. (1996), separated according to Cramer structural class

Endpoint	Class I	Class II	Class III	Sum
Blood effects	3		24	**27**
Body weight changes	15	4	89	**108**
Cardiovascular effects				**0**
Endocrine			4	**4**
Food consumption	4		2	**6**
Gastrointestinal	3	1	6	**10**
Lethal	2	2	9	**13**
Hepatic	1		28	**29**
Immunotoxic				**0**
Musculo-skeletal	2		1	**3**
Multiple effects	31	3	136	**170**
Neurological	1		10	**11**
No effects	47	7	7	**61**
Non-specific effects		1	12	**13**
Ocular			1	**1**
Ovarian			2	**2**
Organ weight changes	11	3	42	**56**
Pulmonary		1		**1**
Renal	7	2	18	**27**
Reproductive	5	2	38	**45**
Spleen			5	**5**
Teratogenic	4	2	10	**16**
Testicular	1		4	**5**
Sum	**137**	**28**	**448**	**613**

The purpose of the present analysis of endpoints was to obtain an overview of which ones most frequently drove the lowest NOEL and whether all the major toxicological endpoints were at least represented in the database. The fact that some endpoints drive NOELs more frequently than others reflects the outcome of the analysis, which generally included more than one study on each substance. It should be noted that the majority of the studies examined multiple endpoints and some endpoints are more frequently affected at the LOEL than others.

None of the NOELs were based on cardiovascular or immunotoxic effects. The absence of cardiovascular effects is likely to reflect the low frequency of such effects as the critical endpoint for chemicals other than pharmaceuticals, and the fact that very few studies in dogs, which would have been more likely to detect cardiovascular effects, were included in the final published database. The absence of immunotoxic effects as a critical endpoint may reflect both the comparatively limited attention paid to this endpoint until recent years as well as the low frequency with which they are

identified as the most sensitive effect for substances showing other toxicities. In none of the rat and rabbit studies was immunotoxicity identified as the critical endpoint determining the NOEL.

In view of the importance of the endpoints of endocrine activity, reproductive toxicity, developmental toxicity and neurotoxicity in relation to TTC values, these are addressed in more detail later (see chapter 4.3).

4.2.3. Assessment of original papers and reports on substances in the lowest 10th percentile of the NOEL distribution

4.2.3.1. Cramer class I substances

The values for the NOELs for all the substances in each of Cramer Class I and Cramer Class III were scrutinised and the substances falling below and around the lowest 10th percentile[8] of the two distributions of NOELs were identified. For these substances, an attempt was made to assess the quality of the critical studies and verify the NOEL values. The lowest 10th percentile was chosen because it includes the substances that determine the TTC values for the respective classes (Munro et al. 1996 derived TTC values by dividing the 5th percentile NOEL by a factor of 100). Any discrepancies in the numerically higher NOELs of the remaining substances above the 10th percentile would have to be substantial to have any impact on the TTC value.

From a total of 137 substances classified in Cramer Class I by Munro et al. (1996), 16 substances below and around the lowest 10th percentile of the distribution of NOELs were examined. Their identity together with the respective NOEL value and critical endpoint(s) determining the NOEL are shown in Appendix C, Table 1. The respective NOEL and cited source were retrieved from Munro et al. (1996). The detailed reasons for non-confirmation of NOELs can be found in Appendix C. Where possible, the original reference for each substance was obtained and reviewed to reach an independent view on its quality and the NOEL. A full search for more recent studies on the 16 substances in Class I (and thus possibly different NOELs) was not performed.

The original papers or reports on the critical studies could only be obtained for 8 of the 16 substances. Thus, the quality of the remaining 8 studies could not be fully assessed. For 6 of the 8 studies not available to us in original form, descriptions of the studies were available from JECFA monographs, most of which identified a NOEL. The other 2 studies were published only as abstracts.

The NOELs used by Munro et al. (1996) were verified, or were judged to be very conservative, for 14 of the 16 substances, when compared with the original study report or JECFA descriptions. In the case of the remaining 2 substances, the findings were as follows: for ethyl acrylate the NOEL identified by Munro et al. (1996) was only slightly higher (by less than one order of magnitude) than the NOEL identified during this evaluation; for 2-phenyl-1-propanol, (listed as Phenyl-1-propanol, 2- in Munro et al., 1996) a NOEL could not be identified in this evaluation as effects on body weight were reported at the lowest dose tested.

For retinol, although the correct NOEL was identified by Munro et al. (1996) from the 1989 study cited of the teratogenic effects of a single dose in pregnant mice, it should be noted that other data available at that time indicated that the NOEL for teratogenicity in the rabbit was lower, by around an order of magnitude (Rosa et al., 1986).

The impact that any adjustments to NOELs might have on the TTC value for Class I substances is difficult to predict from the limited analysis undertaken here. Discarding some of the overly conservative NOELs might move the 5th percentile NOEL upwards. On the other hand, taking account of lower NOELs, including any derived from a scrutiny of more recent data on the same

[8] The number selected is slightly different from the exact 10th percentile because of ties in the ranks of the NOELs.

915 substances, might move the 5th percentile NOEL downwards. Ideally, such an exercise would be done
916 on the entire group of Class I substances. However, based on the present analysis of the lowest 10th
917 percentile of substances, it does appear that the Munro et al. (1996) dataset provides a generally
918 conservative estimate of Class I NOELs.

919 #### 4.2.3.2. Cramer Class III substances

920 From a total of 448 substances classified in Cramer Class III by Munro et al. (1996), 50 substances
921 below and around the lowest 10th percentile of the distribution of NOELs were examined. Their
922 identity and respective NOEL values and the critical endpoint(s) determining the NOEL are shown in
923 Appendix C, Table 2. The respective NOEL and cited source were retrieved from the Munro et al
924 publication. Almost all substances could be identified from the information provided, but in a few
925 cases the name of the substance given was not entirely consistent with the CAS number (e.g.
926 ivermectin), and in one or two cases the CAS number was incorrect or in doubt (trenbolone acetate
927 and 17 α-hydroxytrenbolone).
928
929 In the majority of cases, the cited source was a company report, which had been cited in IRIS and was
930 not retrievable. The NOEL was checked to determine whether (a) it was the critical NOEL for the
931 study cited, and (b) still considered the critical NOEL for the compound, given more recent
932 evaluations, such as by EFSA, JMPR and EPA. It should be noted that this comparison with more
933 recent data was conducted for Class III substances but not for Class I substances because more recent
934 studies for Class III were readily available and the issue was regarded as more critical for Class III
935 substances than for class I substances.
936
937 In general, the NOEL provided by Munro et al. (1996) was the critical NOEL for the cited study, and
938 was numerically correct. In a few instances, a lower NOEL could have been selected (e.g. heptachlor
939 using a different study) or a higher NOEL could have been used, for example because two studies
940 were available and a combined NOEL could have been obtained (e.g. cypermethrin and avermectin
941 B1). In some cases, the NOEL appears to be slightly lower than that cited (e.g. coumaphos, 22,23-
942 dihydroavermectin-B1a - and B1b (ivermectin) and disulfoton). In one case (zeranol) the JECFA
943 summary does not reflect the ovarian toxicity used by Munro et al. (1996). The reasons for this are not
944 apparent from the paper.
945
946 Using current databases and risk assessment criteria, many of the NOELs cited by Munro et al. (1996)
947 would no longer be considered the pivotal NOELs. For example, some endpoints are no longer
948 considered relevant to humans, particularly benign adaptive hepatic hypertrophy (e.g. dieldrin). A
949 major difference is in the assessment of cholinesterase inhibitors. Less weight is now placed on
950 inhibition of plasma cholinesterase. On the other hand, for a number of such compounds, the current
951 NOELs are lower than those given in Munro et al. (1996) (e.g. aldicarb, dichlorvos and fonofos). In
952 the case of acrylamide, this is now considered to be a genotoxic carcinogen, and therefore in
953 retrospect should not have been included in the Munro et al. database.
954
955 Overall, the NOELs analysed here (the lowest 10th percentile as these are likely to be the ones where
956 changes would have the biggest impact on the calculation of the TTC) compared with those cited by
957 Munro et al. (1996) are generally the same or higher, other than for some organophosphates. Thus,
958 from this analysis, the Munro et al. database does appear to provide a conservative assessment for
959 Class III substances, other than for the cholinesterase inhibitors. The case for re-evaluating
960 cholinesterase inhibitors, using the most recent data available, is discussed in section 4.3.2.
961

962 #### 4.2.3.3. Comparison of Munro et al. TTC values with subsequent published
963 data

964 An independent dataset has been utilised (Kalkhof, 2010; Kalkhof et al., 2011) to evaluate the TTC-
965 values derived from the database of Munro et al. (1996). The dataset comprises 861 new industrial

chemicals registered in Europe between 1982 and 2008 selected from the European List of Notified Chemical Substances[9] (NCS) because they have been tested in subacute or subchronic studies. This dataset has no overlap with the database of Munro et al, (1996). The full ELINCS database is available to European Competent Authorities. The analysis was based on the results of 28-day subacute tests conducted according to OECD TG 407 on 776 chemicals. Another 85 chemicals were tested according to OECD TG 408 in 90-day studies. The NOAELs were adjusted to obtain estimated chronic NOAEL values by using a scaling factor of 6 for the results of the 28-day studies and a scaling factor of 2 (ECETOC 1995; ECHA 2010; Kalberlah & Schneider, 1998) for the results of the 90-day studies. This analysis is shown in Table 4 below. Cramer Class II is not included since very few substances were classified in that class. It can be seen that the results of this study support the conservative nature of the TTC values derived by Munro et al. (1996).

Table 4: **Comparison of Munro et al, 1996 5th percentile NOELs with those derived from an EUdatabase on industrial chemicals**

Database	5th percentile NOEL (mg/kg bw per day)	
	Cramer class I	Cramer Class III
Munro et al, 1996	3.0	0.15
EU NCS 28-day	1.7 (65*)	0.8 (691*)
EU NCS 90-day	12.5 (9*)	0.8 (76*)

*Number of chemicals

The Fraunhofer Institute ITEM, Germany, has also published information (Escher and Mangelsdorf, 2009) comparing the 5th percentile NOEL values of Munro et al. (1996) with those derived from a separate database on industrial chemicals, known as the RepDose database (Bitsch et al., 2006; http://www.fraunhofer-repdose.de/). The RepDose database contains oral and inhalational studies in rats and mice on around 600 substances, only 100 of which are common to both RepDose and the Munro et al. database. The majority of the substances in the RepDose database are classified in Cramer Class III (70%), with around 26% in Cramer Class I, and only a few in Cramer Class II. After removing substances in common, they compared RepDose and Munro et al 1996 oral NOELs for Cramer Classes I and III. It should be notes that this comparison was done on a mmol/kg bw per day basis. The results are shown in Table 5 and also support the conservative nature of the TTC values derived by Munro et al. (1996).

Table 5: : **Comparison of Munro et al, 1996 5th percentile NOELs with those derived from the RepDose database**

Database	5th percentile NOEL (mmol/kg bw per day)	
	Cramer class I	Cramer Class III
Munro et al, 1996	0.0115	0.0005
RepDose	0.0357	0.0016

4.3. Adequacy of TTC value in protecting against specific endpoints

4.3.1. Previous evaluations of endpoints of specific concern

The TTC concept and the TOR approach for food contact materials were discussed by the EC Scientific Committee for Food in 1996 and one of the issues raised was whether, for certain endpoints of specific concern, toxic effects might occur at low dose levels which would not be covered by the human exposure thresholds derived by Munro et al. (1996). In particular, concerns were raised about whether effects on the nervous system, immune system, endocrine system and development would be absent at the human exposure threshold values (SCF, 1998). Although the original database published

[9] http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=eli

by Munro et al. in 1996 did include some studies measuring these endpoints of specific concern, they were insufficient in number to provide a robust answer to the question of potential low-dose effects.

An Expert Group was therefore set up by ILSI Europe to examine this question in more detail (Kroes et al., 2000). Expanded databases were developed for the toxicological endpoints of neurotoxicity (82 substances), immunotoxicity (37 substances), developmental neurotoxicity (52 substances) and developmental toxicity (81 substances). They were analysed to see if toxic effects involving these endpoints occurred at lower doses than those for structural Cramer Class III substances in the original database of Munro et al. (1996). The analysis showed there was no difference between the cumulative distributions of NOELs for Cramer Class III substances and those for the four selected endpoints, other than for neurotoxicity. The cumulative distribution of NOELs for neurotoxicity was not only lower than those of the other selected endpoints, but it was also clearly lower than that for structural Cramer Class III substances . Consistent with the earlier findings of Cheeseman et al. (1999), the TTC value of 1.5 µg/person per day, based on cancer endpoints, covered all these effects, being 2-3 orders of magnitude lower than the neurotoxicity NOELs divided by a safety factor of 100.

Subsequently Kroes et al. (2004) further explored whether particular neurotoxicants should be considered as a separate class. Using the expanded database from the earlier work (Kroes et al., 2000) and locating the most sensitive indicators of effects that they could find, the NOELs for the most potent neurotoxicants, the organophosphorus compounds (OPs), were plotted separately from the other neurotoxicants. They noted that the 5th percentile NOEL for OPs was lower, by around an order of magnitude, than the corresponding 5th percentile NOEL for other neurotoxicants. The other neurotoxicants resulted in a plot comparable to the Cramer Class III chemicals examined by Munro et al. (1996). By applying a safety factor of 100 to the 5th percentile NOEL for OPs, Kroes et al. (2004) derived a human exposure threshold of 18 µg/person per day (Table 6) and recommended that this figure be used for OPs rather than the value of 90 µg/person per day used for other substances in structural Class III.

Table 6: Human exposure threshold value for organophosphates from Kroes et al., 2004

Structural class	Fifth percentile NOEL (mg/kg bw per day)	Human exposure threshold (µg/person per day)
Organophosphates	0.03	18

4.3.2. Anti-cholinesterase-related neurotoxicity endpoints

The Committee investigated whether the proposed TTC value for OPs of 18 µg/person per day (corresponding to 0.0003 mg/kg bw per day) adequately covers neurotoxic effects of substances with anti-cholinesterase (AChE) activity. An analysis was undertaken using the comprehensive EFSA internal database on pesticides. Article 41 of Regulation (EC) 369/2005 on maximum residue levels requires EFSA to develop, maintain and continuously update a database containing toxicological reference values, i.e. acute reference doses (ARfDs) and acceptable daily intakes (ADIs) for active substances in pesticides for which maximum residue levels (MRLs) have been established. Listed are reference values established by the European Commission (COM), the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), European Member States, the European Community Co-ordination Peer Review Meetings (ECCO), and EFSA and its Pesticide Risk Assessment Peer Review Unit (PRAPeR). For a number of active substances, more than one ADI/ARfD has been established. Notably, this pesticide database also contains a significant number of active substances belonging to the chemical classes of OPs and carbamates, which cause inhibition of AChE, a mechanism leading to neurotoxicity at low doses, and consequently also to establishment of low ADIs.

In order to investigate if and to what extent the ADIs of highly potent neurotoxic substances (i.e. AChE inhibitors) are lower than the proposed TTC value for OPs , the ADIs of all OPs and carbamate

pesticides in the database (status as of 6th May 2010) were extracted and compared with the proposed TTC value of 18 µg/person per day (equivalent to 0.3 µg/kg bw per day).

The ADIs for OPs and carbamates that are listed in the database and are shown in Table 1 in Appendix D. From Table 1, Appendix D, substances with ADIs at or below the proposed TTC value for OPs were extracted and are listed in Table 2 of Appendix D.

In summary, for 59 OPs and 14 carbamates, 93 and 27 ADIs have been retrieved, respectively. Out of the 93 ADIs established for OPs, 83 were above the proposed TTC value, 7 were at the proposed TTC value, and only 3 were below the proposed TTC value (i.e. the ADIs for diazinon, mevinphos and prothiofos). For the 14 carbamates, only one ADI was below the proposed TTC value (i.e. one out of the 3 ADIs for carbofuran). Given that the TTC is a probabilistic approach, the present analysis on OP and carbamate ADIs confirms the validity of the proposed TTC value for inhibitors of AChE of 18 µg/person per day (equivalent to 0.0003 mg/kg bw/day) and establishes that it can be applied to both OPs and carbamates. Although some of the critical effects listed in Table 2, Appendix D, cannot be definitely attributed to neurotoxicity, critical effects on brain AChE are included and this analysis shows that the TTC of 18 µg/person per day would be protective.

4.3.3. Reproductive and developmental toxicity

Reproductive toxicity deserves specific consideration in the context of the TTC concept as it has unique features as compared to other forms of toxicity. Infertility and birth defects are considered by society as severe adverse effects that may require dedicated preventive measures. In the REACH legislation, reproductive toxicity is grouped with carcinogenesis and mutagenesis in needing specific restriction and authorization. Reproductive toxicity can be expressed in many different manifestations dependent on the nature, timing, duration, and magnitude of exposure relative to the phase of the reproductive cycle and has many different underlying mechanisms. It is therefore difficult to group reproductive toxicants in a single analysis. One analysis has combined developmental toxicants based on published literature of *in vivo* reproductive and developmental toxicity studies (Kroes et al., 2004). It was concluded from that analysis, albeit limited, that more stringent TTC values than those applied for non-cancer endpoints would not be necessary to protect against reproductive and developmental toxicity. Subsequent analyses on 91 substances assessed under the EU existing chemicals programme (Bernauer et al., 2008) and on 93 industrial chemicals tested by BASF (van Ravenzwaay et al., 2011) came to similar conclusions.

The approach followed here by the Scientific Committee was to analyse the applicability of the TTC concept for those substances carrying an EU classification for reproductive and/or developmental toxicity. Substances classified according to Directive 67/548/EEC by the European Union for developmental toxicity (category 1, 2 or 3) or effects on sexual function and fertility (category 1, 2 or 3) were selected. The analysis was performed on 85 developmental toxicants (chemicals classified with EU risk phrases R61[10] or R63[11]) and 54 fertility toxicants (chemicals classified with EU risk phrases R60[12] or R62[13]). Using the Toxtree software version 2.1.0 (http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE) to generate Cramer classifications (by the classical Cramer scheme), it was found that the majority of chemicals were placed in Cramer Class III, followed by Cramer Class I. Very few chemicals were classified into Cramer Class II. In the case of the developmental toxicity dataset, the breakdown of chemicals by Cramer class was: 71 Cramer Class III, 1 Cramer Class II and 13 Cramer Class I. In the case of the fertility toxicity dataset, the breakdown was: 43 Cramer Class III, 3 Cramer Class II and 8 Cramer Class I.

[10] May cause harm to the unborn child.
[11] Possible risk of harm to the unborn child.
[12] May impair fertility.
[13] Possible risk of impaired fertility.

Chemicals from the developmental and fertility datasets were merged (102 reproductive toxicants) and divided into two subsets of chemicals, classified into Cramer Class I or Cramer Class III, and the NOEL value distributions were analysed. Since the 5th percentiles of the NOEL distributions for the Cramer Class I and Cramer Class III subsets in this analysis (Table 7) are higher than the corresponding 5th percentile values for Cramer Class I and Cramer Class III calculated by Munro et al. (1996), it can be concluded that the TTC values derived by Munro et al. (1996) are protective for developmental and fertility effects, assuming that substances carrying classification for reproduction are likely to represent a worst case scenario among those substances that have been tested for reproductive effects. The Scientific Committee is aware that there is likely to be overlap in the dataset used in the analysis above and those of Bernauer et al. (2008) and van Ravenzwaay et al. (2011). This analysis confirms the conclusions of the previous studies referred to above.

Table 7. Cumulative distribution analysis of a dataset of substances classified on the basis of developmental and fertility toxicity

Structural Cramer Class	No. of chemicals (developmental + fertility NOEL)[14]	Calculated 5th percentile NOELs derived in this analysis (µg/kg bw/day)	5th percentile NOELs from Munro et al. (1996) (µg/kg bw/day)
Class I	15	3840	3000
Class II	4		
Class III	83	550	150

Data provided by RIVM (André Muller, RIVM, personal communication)

4.3.4. Substances with endocrine-modulating activity

Consideration of substances with the potential for endocrine-modulating activity is relevant since some have concluded that the TTC approach might not be applicable because of the uncertainty about low-dose effects (Kroes et al., 2004). The hazard assessment of substances with endocrine-modulating activity has been an issue of extensive debate over recent decades. For humans, concerns in this area include reproductive organ development, reproductive function and effects on the hypothalamic-pituitary-thyroid axis. Also, the glucocorticoid, insulin and neuroendocrine systems have been mentioned in relation to substance-mediated endocrine modulation. The relevance of such findings from *in vitro* and animal studies for human hazard and risk assessment of endocrine active substances is currently being discussed extensively in the scientific community (EFSA, 2010b).

Many xenobiotic chemicals have been shown to have endocrine-modulating activity in various *in vitro* systems in which, for example, receptor binding and receptor activation are determined. However it is important to distinguish these kinds of interactions from adverse effects at the level of the organism. Indeed, the consolidated definition of an endocrine active substance agreed at the 1996 *European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife* (EC, 1997) states that it "is an exogenous substance…that alters function(s) of the endocrine system, causing adverse health effects in an intact organism, or its progeny…" . Apart from pharmaceuticals with endocrine activity by design, the potency of endocrine-active xenobiotics is often several to many orders of magnitude lower as compared with the activity of endogenous hormones. Therefore, to achieve endocrine mediated effects *in vivo*, usually higher doses have to be administered compared to the exposures expected to occur from dietary or environmental sources. Attempts to increase the sensitivity of *in vivo* assays for endocrine activity have been only partly successful. This is probably attributable to the plasticity of endocrine homeostasis, which is characterised by a high level of

[14] Some substances have been classified both for fertility and developmental endpoints.

compensatory feedback. In the recent updating of the OECD Test Guideline (TG 407) for repeated-dose 28-day oral toxicity study in rodents, after testing a series of proposed endocrine parameters, only the thyroid hormones were found useful and were included in addition to classical histopathology of endocrine organs (OECD, 2006). Other endocrine parameters were deemed too variable and insensitive and therefore less informative for hazard identification.

The issue of low-dose effects of substances with endocrine activity has also given rise to extensive debate. This has been caused in part by the absence of reproducibility of reported low-dose effects in experimental animal studies. In addition, the discussion on the human relevance of these effects has also focused on the question of adversity. Endocrine exposures are handled by the body primarily by adaptive homeostatic mechanisms. Only if the body is unable to regulate exposures within its limits of homeostasis is the threshold of adversity crossed. In that case, adverse effects can occur, which is often referred to as endocrine disruption. Endocrine disruption-related toxicity may have specific features deserving special attention (e.g. high susceptibility of long-term developmental programming). However, such features have been assessed extensively and comprehensively in hazard and risk assessment procedures that were in place when the Munro et al. (1996) database was compiled.

Current knowledge supports the proposition that existing TTC values will also cover many endocrine-mediated adverse effects, particularly those involving reproduction, development and thyroid function. For example the analysis of the more recent data on reproductive toxicity (see 4.3.3.) was based on data using existing, globally harmonized test protocols such those for repeated-dose toxicity (OECD TG 407, 408, 409), and chronic toxicity and carcinogenicity (OECD TG 452, 453), and especially those for reproductive and developmental toxicity (OECD TG 414, 415, 416). The two-generation test (OECD TG 416) is currently considered the critical test for hazard assessment of most endocrine parameters. The Scientific Committee notes that the Munro et al. (1996) database included a number of studies in which these endpoints were evaluated. Subsequent analyses in this opinion (see section 4.3.3.) and in the published literature (Kroes et al, 2004; Bernauer et al, 2008; van Ravenzwaay et al., 2011) also indicate endocrine-mediated adverse effects are likely to be covered by the existing TTC values.

4.4. Substances not suitable for the TTC approach

It is necessary to consider whether it may not be appropriate to apply the TTC approach to certain categories of substances. Several categories for exclusion have already been identified by Cramer et al. (1978) and Kroes et al. (2004) as indicated below.

4.4.1. Categories previously recommended for exclusion by others

4.4.1.1. Inorganic substances

Inorganic substances should be excluded as they are not represented in the Cramer et al. (1978) decision tree on the three structural classes, nor are they represented in the toxicity database of Munro et al. (1996).

4.4.1.2. Metals

There is a wealth of information in both animals and humans on the toxicity of many of the heavy metals, such as arsenic, cadmium, lead and mercury. In addition, metals are not represented in the Cramer et al. (1978) decision tree on the three structural classes, nor are they represented in the toxicity database of Munro et al. (1996). Some metals, such as cadmium and lead, also bioaccumulate. For these reasons it was recommended by Kroes et al. (2004) that the TTC approach should not normally be applied to non-essential metals in elemental, ionic or organic forms.

4.4.1.3. Polymers

Cramer et al. (1978) recommended that polymers should be excluded because they are not structurally defined in terms of chain length, molecular weight and cross-linking.

4.4.1.4. Certain substances that bioaccumulate

Kroes et al. (2004) recommended that substances with extremely long half-lives that show very large species differences in the extent of bioaccumulation, such as TCDD and its structural analogues should be excluded. In their decision tree, they specifically excluded polyhalogenated-dibenzodioxins, -dibenzofurans and –biphenyls.

4.4.1.5. Proteins

Proteins were recommended for exclusion by Kroes et al. (2004) because of the possibility of allergenicity at low exposures. In their view, a specific TTC value would need to be developed to cover the endpoint of allergenicity once sufficient low-dose response data were available. Proteins were not included in the Munro et al. (1996) database, although proteins that are common components of food would be classified as Class I or Class II substances under the structural decision tree of Cramer et al. (1978).

4.4.1.6. Substances with endocrine activity

Kroes et al. (2004) considered that there were a number of important uncertainties surrounding low-dose effects of substances with endocrine activity and, by implication, the TTC approach should not be applied to a substance known to have such activity.

4.4.1.7. High potency carcinogens

Kroes et al. (2004) recommended that the TTC approach should not be applied to aflatoxin-like, azoxy- or N-nitroso-compounds. This is because for these substances the upper bound lifetime risk for cancer is greater than one in a million even at an exposure of 0.15 µg/day (the TTC value for substances with a structural alert for genotoxicity).

4.4.2. EFSA considerations of categories previously recommended for exclusion and recommendations for additional exclusions

The Scientific Committee agrees that the categories mentioned in 4.4.1. above are not suitable for the TTC approach, with the exception of substances with endocrine-mediated activity, which have been addressed in section 4.3.4. of this opinion.
The Committee also considers that, while metals in organic form are generally to be excluded from the TTC approach (see 4.4.1.2.), organic salts, where the counter ion is an essential metal, may be suitable for the TTC approach.

The Scientific Committee notes that EFSA currently evaluates polymers and oligomers by read-across from the respective monomers on which there are toxicity data. In addition to the exclusion categories mentioned in 4.4.1. (with the exception of substances with endocrine-mediated activity), the Scientific Committee also recommends that the TTC approach is not suitable to be applied to substances in the following categories:

4.4.2.1. Other substances with a high potential for bioaccumulation

Two situations are relevant to TTC:

- bioaccumulation as a result of direct chronic exposure.
- bioaccumulation with possible bio-magnification in food species and its implications for exposure assessment.

Chronic exposure studies in experimental animals inevitably incorporate bioaccumulation potential in that species. The majority of the substances studied have been either polyhalogenated aromatic compounds or metals. A limited number of polyhalogenated hydrocarbons are in the current TTC databases. An important question is whether there is any correlation between bioaccumulation and toxic/ carcinogenic potency. It is evident from the lifetime cancer risk calculations that a number of these substances are more potent than the TTC values for cancer endpoints, but also that this is often not the case. Thus as a conservative approach, any available information on properties that are associated with bioaccumulation should be considered specifically as part of the TTC approach.

There are two options both of which require the prior identification of substances with likely bioaccumulation properties:

- to eliminate from further consideration those substances that are predicted to have bioaccumulation properties, or
- to incorporate a specific allowance for those substances with properties associated with bioaccumulation.

The structural and physico-chemical properties that facilitate bioaccumulation appear to be:

- high octanol-water partition coefficient (e.g. between 3.5 to 9), resulting in a tendency to be retained in the body due to concentration in tissues such as adipose tissue, and/or a tendency for re-absorption,
- marked steric hinderance of metabolism (occupancy of the most likely sites of metabolism by animals/ gut microflora),
- high stability of chemical bonds.

4.4.2.2. Substances with structures that are not adequately represented in the original databases

The original databases underpinning the TTC approach (Cramer et al., 1978; Munro et al., 1996; Cheeseman et al., 1999; Gold et al. 1984 and its subsequent updates) contain a large number of substances covering a wide range of chemical structures, a wide range of technical functions and a wide range of toxicological endpoints. However, if the TTC approach is to be applied in a new area, it is important to consider whether the original databases are sufficiently representative of the substances in the new area, from the perspective of chemical structures. The Scientific Committee considers that the technical function *per se* of a substance is irrelevant, thus it does not consider that lack of substances in the original databases with a particular technical function excludes application of the TTC approach to that technical area, provided that the strcutural features are adequately represented (see chapter 4.8).

4.4.2.3. Substances predicted to have the potential for local effects on the gastro-intestinal tract

If a substance has physico-chemical properties or data indicating the potential for local effects (e.g. irritancy or corrosion) on the gastrointestinal tract or it has a structural similarity to a substance known to exert local toxic effects, it should be excluded from the TTC approach.

4.4.2.4. Nanomaterials

For nanomaterials, in either natural or engineered form, there is not sufficient toxicity information available to investigate whether they would exhibit toxicity directly attributable to their nanoform at exposures below the existing TTC values (EFSA, 2009, 2011). Accordingly, they should be excluded from the TTC approach at present.

4.4.2.5. Radioactive substances

Radioactive substances should be excluded from the TTC approach since they may induce adverse effects by mechanisms due to their radioactive properties (i.e. physical mechanisms) which are different from the adverse effects that may arise from the chemical properties of the substance.

4.4.2.6. Essential elements

These should be excluded because there is a physiological requirement for essential elements, such as selenium, sodium, calcium, and tolerable upper intake levels (ULs) have already been established in most cases by the Scientific Committee on Food (SCF) or by EFSA's Scientific Panel on Dietetic products, Nutrition and Allergies (NDA Panel).

4.5. Adaptation of the TTC values for infants and children

Concern has been raised about the fact that the TTC values of 1800, 540, and 90 µg per person per day for Cramer Class I, II, and III substances, respectively, are expressed on a per person (60 kg adult) basis and these may not be adequately protective for infants and children due to their lower body weights. Other concerns brought forward are the fact that infants and children, on a per kg body weight basis, have a higher food intake than adults, and also have other dietary habits and food preferences, and therefore it is important to take these into consideration when making exposure estimates for the TTC approach. In addition, infants and children are often assumed to be potentially more sensitive to (some) toxicological insults than adults.

Potential differences between infants or children and adults in dietary exposure and susceptibility to chemicals were addressed at an ILSI Europe Workshop on the Applicability of the ADI to infants and children (Clayton et al., 1998). The considerations on the applicability of the ADI raised in this Workshop are also of value for the consideration of the TTC values of the three Cramer structural classes for infants and children.

According to the original premises defined by the JECFA and the SCF, the appropriately assigned safety factor used in the derivation of an ADI is intended to cover differences in species sensitivity, synergistic or antagonistic actions among food additives and other components of food, the heterogeneity of the exposed human population with regard to pregnancy, physiological status and nutrition, age differences between exposed individuals and the variability in susceptibility with age to the potential adverse effects of an ingested chemical substance. The default safety factor of 100 has later been rationalised as comprising a factor of 10 for interspecies differences (most sensitive animal species to humans) and 10 for inter-individual differences between humans. These two 10-fold components of the safety factor can also be subdivided in such a way to allow separately for differences in toxicokinetics and toxicodynamics (WHO, 1999a).

From examination of the differences in toxicokinetics, the ILSI Workshop found that the elimination/clearance of xenobiotics in children is either similar or, in many cases, higher than in adults. In consequence, children frequently will have a lower body burden than adults for the same daily intake of a chemical when expressed on a body weight basis. Based on this, the ILSI Workshop concluded that an increased safety factor was not required for differences in toxicokinetics between post-suckling infants or children and adults.

However, the Workshop emphasised that this conclusion does not apply to neonates and infants before the age of 12 weeks during which period the maturation of xenobiotic metabolising enzymes and elimination processes, such as renal excretion, take place.
Several newer studies support the immature status of xenobiotic metabolising enzymes and elimination processes in newborns up to the age of 3-6 months (De Zwart et al., 2002; Abraham et al., 2005; Dorne & Renwick, 2005; Mielke & Gundert-Remy, 2009). The Scientific Committee also considered the situation of infants of less than 6 months. At birth, renal function has a reduced capacity to excrete substances into the urine, characterised by a renal clearance of 30% to 50%

compared to adults. In the first weeks of life, renal function gradually increases to a functional status comparable to the adult. Similarly, the expression level of some phase-I and phase-II enzymes is 10% to 50% of adult level, which results in a relatively slow elimination of substances in the first months of life. Thus, the metabolic capacity gradually reaches adult levels within the first half year of life. This physiological pattern leads to higher internal exposure as compared to children of more than 6 months and to adults. For some substances, this might result in higher toxicity at the same level of external exposure. The Scientific Committee noted that reduced elimination and excretion is transient and that the toxicokinetic differences between young infants and children or adults is generally not more than 2 to 5 fold (Renwick et al., 2000). In cases where the critical exposure group under consideration is young infants and the estimated exposure is in the range of the TTC value, careful consideration needs to be given to whether the TTC approach should still be applied, also taking into account additional uncertainties due to toxicodynamic differences between species at very young ages.

4.6. Expression of TTC values on a body weight basis

In considering whether the TTC values should be expressed on a per person basis or a per kg body weight basis the Scientific Committee noted that the TTC approach is related to life-long exposure, including exposure during early infancy. Bearing in mind that the low bodyweights of infants and children could have a significant impact on systemic exposure to a substance present in the diet, the Scientific Committee concluded that the TTC values should be converted to a µg/kg body weight basis for comparison with exposure estimates for different age groups. This is shown in Table 8 below.

Table 8. Conversion of TTC values into µg/kg body weight[15] per day

Type of TTC value	TTC value in µg/person per day	TTC value in µg/kg bw per day
With structural alert for genotoxicity	0.15	0.0025
OPs and carbamates	18	0.3
Cramer Class III	90	1.5
Cramer Class II	540	9.0
Cramer Class I	1800	30

4.7. Genotoxicity prediction tools

In working though the TTC decision tree, it is necessary to assess the potential for genotoxicity. Traditionally, the set of structural alerts originally defined by Ashby and Tennant (1991) has been used. Since then a wide range of software tools have become freely and commercially available for the qualitative prediction of potential genotoxicity and genotoxic carcinogenicity. Some of these are based on more extensive lists of structural alerts than the Ashby alerts. The current status of software models has been reviewed recently (Serafimova et al., 2010), and the applicability of selected models in predicting the genotoxic potential of pesticides has been evaluated (Worth et al., 2010). In general, the models are either based on expert knowledge, including structural alerts (molecular substructures) associated with genotoxicity and/or carcinogenicity, or they are based on statistical models which use molecular descriptors as predictor variables. Some are so-called hybrid models, based on a combination of expert rules and statistical models. For the most part, available models are based on potential chemical reactivity with DNA and are comparable in performance to the Ames test (Benigni et al., 2010). Relatively few models accurately predict the results of *in vivo* genotoxicitytests , and few

[15] Based on the fact that the orginal TTC values were calculated for 60 kg adult.

models explicitly capture molecular mechanisms other than DNA reactivity (e.g. covalent binding to proteins, and non-covalent interactions with DNA and protein). It is outside the scope of this document to give guidance on which specific QSAR tools are fit-for-purpose and further work is needed in this area. However, a range of key principles are commonly applied when assessing the adequacy of model prediction (Worth et al, 2010). In particular, it is useful to demonstrate that the model is applicable to (gives reliable predictions for) the class of chemical being predicted.

4.8. Metabolic prediction tools

In the JECFA and EFSA TTC procedures for flavourings (see Appendix B), the predicted metabolic fate of a substance is an important consideration, with consequences for how the different TTC values are applied. Those substances predicted to be metabolised to innocuous products, based on toxicity data on the predicted metabolites or related substances, are evaluated using the so-called A-side of the decision tree, whereas if metabolites are not predicted to be innocuous they are evaluated using the B-side (Renwick, 2004). In this context, JECFA (WHO, 1997) has defined "innocuous products" as "products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agents". JECFA advises that predicting metabolism is difficult and depends very much on expert judgement. There is no evidence from their published monographs that JECFA has used metabolic prediction tools for this purpose.

The issue of metabolic fate as discussed above appears to be relatively specific to the evaluation of flavourings by JECFA and EFSA, possibly because the structures of many of the compounds make such predictions possible and there is information available on metabolism and the toxicological properties of the products or related substances. However, in more generic approaches to the application of the TTC (e.g. Kroes et al., 2004; Renwick, 2005), there is no such consideration of metabolism.

One area where some consideration of metabolic fate may contribute to the application of the TTC approach more generally is that of genotoxicity. In addition to the possible genotoxicity of the parent substance, the potential for metabolism into a genotoxic product must be considered. As genotoxicity is one of the few endpoints where conclusions on toxicological relevance are based more on qualitative than on quantitative grounds, metabolic prediction might have some potential utility here. In the absence of specific information, all TTC schemes require some early consideration of the potential for genotoxicity. A number of reviews have assessed the predictability of metabolic fate, using metabolic prediction tools. The general conclusion is that qualitative prediction is often possible, i.e. the profile of metabolites that will be formed, although it is sometimes difficult to set the stringency (probability constraints) during the prediction such that the complexity of the metabolic fate of a compound is not either over- or under-predicted. In addition, quantitative prediction, i.e. the quantities of the individual metabolites that will be formed, remains elusive. In the absence of such information it is not possible to estimate exposure as accurately as would be necessary for effective application of the TTC approach for non-genotoxic compounds. One option might be to use the TTC for the highest Cramer class from amongst those of the parent and all predicted metabolites, with suitable constraints on their probability of formation, for comparison with predicted or estimated exposure to the parent plus metabolites.

Metabolic prediction has been reviewed recently under an EFSA contract relating to the work of the EFSA PPR Panel. Further information can be obtained from the report of that evaluation (EFSA, 2010a). Most of the programs available for metabolic prediction are commercial and hence there is an underlying cost in their application. The predictive output from the programs would have to be input to another package to predict likely genotoxicity. In some cases, the respective programs have an integrated interface, so that the process is relatively seamless.

More information on metabolic prediction tools can be found in reviews (Boobis et al, 2002; Kulkarni et al, 2005; Norinder & Bergström, 2006; Mostrag-Szlichtyng & Worth, 2010a, b). However, further work in this area is needed for practical application to the TTC approach.

4.9. Chemoinformatic analysis of TTC datasets

During 2010-2011, an EFSA-funded study (Bassan et al., 2011) was carried out by an external contractor. The goals of the project were:

1) To assess whether the chemical structures in the two main datasets underpinning the TTC approach (the Munro et al. and CPDB datasets) were adequately representative of chemical space and therefore of the 'world of chemicals' in general.
2) To critically evaluate the Cramer scheme on classification of chemical structures to assess whether it is robust.
3) To explore whether the TTC approach could be refined and improved by incorporating physicochemical data (experimental and computed) or toxicity data generated by non-testing methods such as Quantitative Structure-Activity Relationships (QSARs).

In order to undertake these analyses, the Munro et al. and CPDB[16] databases were compiled into two new electronic datasets (freely available from the EFSA website[17]), including quality-checked chemical structures, toxicity data (NOEL and TD_{50} values, respectively), and a wide range of calculated molecular descriptors encompassing both structural features and physicochemical properties. Chemical space analysis was performed by the use of chemoinformatics methods, including Principal Components Analysis (PCA), Cluster Analysis, Soft Independent Modelling of Class Analogy (SIMCA) and Partial Least Squares (PLS).

For the investigation of whether the two main TTC databases are representative of the world of chemicals, the chemical space occupied by the structures within each dataset was investigated and each dataset was also compared with a subset of 502 chemicals drawn randomly from the Distributed Structure-Searchable Toxicity (DSSTox) Database compiled by the US Environmental Protection Agency. The DSSTox Database[18] contains approximately 10,000 substances in total, including industrial chemicals, pesticides, consumer chemicals and food-use chemicals. This database is considered to be broadly representative of the "world of chemicals". The TTC datasets were also compared with another subset from the DSSTox Database, defined as "food-use" chemicals (food additives and food contact substances). The results of this analysis were as follows.

1) The Munro et al. and CPDB datasets can be clustered into subgroups, where the individual subgroups have more homogeneous structural characteristics (e.g. degree of branching, globularity, number of ring atoms) than the original datasets.
2) The Munro et al. and CPDB datasets are overlapping in chemical space, and are broadly representative of the universe of chemicals, as demonstrated by comparison with the random dataset from the DSSTox Database.
3) The Munro et al. dataset includes a higher proportion of high molecular weight substances than the DSSTox subset of food-use chemicals.
4) The CPDB dataset includes a higher proportion of polyaromatic compounds than the DSSTox subset of food-use chemicals.

To explore the possibility of developing models for the quantitative prediction of chronic toxicity (NOEL values) and carcinogenic potency (TD_{50} values), correlation analysis, PLS and ranking methods were applied (Pavan & Todeschini, 2008, 2009: Pavan & Worth, 2008). The results indicated that no predictive QSAR models could be developed for the Munro et al. and CPDB datasets with respect to NOELs or TD50s. However, the results of ranking analysis, based on molecular descriptors, indicated that trends can be established and used for interpolation between a substance of

[16] New compilation of the CPDB dataset, developed and donated by Dr Chihae Yang (USA)
[17] http://www.efsa.europa.eu/en/supporting/pub/159e.htm
[18] http://www.epa.gov/ncct/dsstox/

unknown toxicity and substances with similar molecular descriptors and known toxicological properties. This enables a semi-quantitative prediction of the NOEL to be made.

In the critical evaluation of the Cramer classification scheme, the results of the analysis showed that:

1) For the structures in the Munro et al. database, the Cramer scheme is highly conservative and performs better in identifying high hazard substances than low hazard ones.
2) The Cramer scheme also performed well in classifying chemical structures into hazard classes from the CPDB dataset (for which it was not specifically designed); the majority of carcinogenic substances (409 out of 461) were classified into Cramer Class III, as were the majority of substances that were positive in the *Salmonella* assay (266 out of 279). In other words, the Cramer scheme was found to be conservative when applied to a large majority of carcinogens in the CPDB, not all of which are genotoxic, and is thus broadly protective not only for chronic toxicity but also for carcinogenicity.
3) The Cramer classification scheme, when applied to the Munro et al**.** dataset, could be slightly improved by combining it with a ranking classification model which utilised the molecular descriptors most closely correlated to chronic toxicity (NOEL values); this indicates that statistically-based methods and molecular descriptors encode some useful information not already included in the Cramer rules.
4) However, none of the classification schemes developed in the project, using a wide variety of statistical methods and molecular descriptors were significantly better than the Cramer scheme.
5) The Cramer scheme provides a conservative means of classifying substances on the basis of chronic toxicity NOELs.

Finally, statistical analysis of the TD_{50} values in the CPDB showed that mutagenic (*Salmonella* positive) substances tend to have higher carcinogenic potencies (their TD_{50} values are around 6.5 times lower) than non-mutagenic (*Salmonella* negative) chemicals. This confirms an earlier, similar analysis by Cheeseman et al. (1999) and supports the usefulness of incorporating genotoxicity alerts into an overall TTC scheme and the lower TTC human exposure value for substances with such alerts.

Overall, the results of the study confirm that the Munro and CPDB databases are broadly representative of the world of chemicals. They confirm the protectiveness of the Cramer scheme for both non-cancer and cancer endpoints. They also indicate the potential of modern chemoinformatics methods for exploring relationships between chemical structure and toxicity, indicating these methods could be useful in the future for developing alternative hazard classification schemes associated with TTC values.

4.10. Exposure

The application of the TTC concept depends on an exposure assessment in which there is confidence that it is not an underestimate. This is done using different methods depending on the substance of interest. Opinions giving an overview of exposure assessment methods and their uncertainties have been published by EFSA (EFSA, 2005b, 2006). Information on the methods used by the various EFSA Panels is given in Appendix E.

4.10.1. Dietary intake estimates for TTC

4.10.1.1. High exposure estimates

It is essential for application of TTC to have a good estimate of high exposures. It is usual practice in most of the EFSA Panels to use mean and high percentile food consumption (e.g. 95th percentile) and average chemical concentration values, measured or predicted, to estimate chronic dietary exposure of average and high consumers. In other Panels, maximum predicted concentrations in food are used, sometimes in conjunction with a standard food basket. In some Panels, acute exposure (24 hours or

less) is also considered using different methodology It may also be important to consider exposure in specific population subgroups as for example infants and children.

Such methods are often criticised for being overly conservative, especially for consideration of lifetime exposure, and as for all point estimates can only be used with one concentration per food item, but such conservative methods are appropriate for initial comparison with TTC values. Given the conservatism of these estimates, it may be concluded that if the TTC value is not exceeded, then further analysis of toxicity or exposure is not necessary. If the TTC value is exceeded, then a more refined approach for exposure assessment may be appropriate, along with other considerations such as the possible need for chemical-specific toxicity data. For some types of chemicals the conservative high exposure estimate is obtained by using maximum predicted exposure, from different food categories. This is only feasible for substances that have a preregistered use such as additives and flavourings, where the concentration in the food is known. For most substances, the maximum possible level (MPL) in food groups is combined with standard consumption figures for those food groups to give a predicted maximum exposure. This also provides a level of conservatism, for which the same principle applies as for the use of food baskets. For contaminants, normally results from chemical analysis of foods are used to estimate exposure .

4.10.1.2. Refinement of dietary exposure estimates

If it is considered that the exposure estimate should be refined, this can be done with different approaches but in most situations when the TTC is applicable, it is possible that there will be insufficient data to make such refinement. At the moment, the most refined method for assessing dietary exposure is probabilistic exposure assessment. This method combines random sampling from the available occurrence data and from food consumption data, which results in a prediction of the probability of different exposure levels in the population. It takes into account all measured levels of the substance (also below the limit of quantification, see also EFSA, 2010c) and all volumes of consumption, including from multiple food sources.

A drawback of this method is that it requires high quality input data, i.e. adequate occurrence data as well as national data on food consumption. Also it requires considerable infrastructure and expertise to perform. Detailed national consumption data are being gathered in the EFSA Comprehensive European Food Consumption Database, with figures on national consumption at individual food or food group level (EFSA, 2011a[19]). The intention of the database is to provide a refined tool for EFSA, its Scientific Panels, and potentially for other scientists in European Member States, to allow detailed estimates of consumers' exposure.

4.10.2. Duration of exposure

The NOELs used for the development of TTC values have been derived from chronic studies, or from sub-chronic studies with a scaling factor of 3 applied to convert sub-chronic NOELs to chronic NOELs. Exposure to substances in food or feed in the working field of EFSA will generally be of a chronic nature. However, there may be situations where a short-term or intermittent exposure period may be considered, such as incidents or presence of a substance during time-limited production period. The TTC approach may be applicable in these situations. Some authors have proposed alternative methods for applying the TTC approach to short-term exposures in the area of pharmaceutical impurities (Müller et al., 2006), cosmetics (Kroes et al., 2007), and trace chemicals in food (Felter et al., 2009).

Felter et al. (2009) proposed that there are two ways in which short-term exposures might be addressed. The first is to modify the exposure assessment to determine an equivalent daily exposure. This kind of an approach was recommended by Kroes et al. (2007) for evaluating exposures

[19] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm?wtrl=01

associated with cosmetics that are not used on a daily basis. The Scientific Committee notes that this requires fairly robust data on the nature of the exposure and its duration.

A second approach would be to establish TTC-based limits for short-term exposure durations that are less well-defined. An example of this might be that a substance in food is only present for a few months; protection for lifetime exposure is then overly conservative. This was the rationale used by Müller et al. (2006) to establish different TTC tiers for genotoxic impurities in pharmaceuticals corresponding to different exposure durations. The basis for this approach comes from the use of lifetime cumulative dose (Felter et al., 2009). They referred to Haber's law (concentration * time = constant [toxicity]) but also indicated that this is a simplified representation of the processes leading to toxicity.

Although the proposals above have been put forward, the Scientific Committee is not confident about the general applicability of these proposals and also notes that the current TTC values are derived from databases that do not address effect from acute exposure. It is therefore recommends that the issue of less than chronic exposure should be addressed case-by-case. This could be done for example by considering the margin between the appropriate TTC value (without any adjustment for duration of exposure) and the estimated dietary intake.

4.11. Routes of exposure other than oral

Most of EFSA's risk assessment work relates solely to oral exposure to substances via food and feed. However, some Panels have included in their remit the requirement to assess exposure of users/workers to the same substances by other routes, such as those working with pesticides or substances added to animal feed. Thus, consideration of the applicability of the TTC approach to substance exposure by routes other than the oral route is relevant to the work of EFSA.

4.11.1. Existing databases for TTC – non-cancer endpoints

The database of Munro et al. (1996) consists of 613 chemicals for which over 2900 NOELs have been compiled. For 549 of the chemicals, the route of exposure was oral with more than half of these (344) by dietary input. In 62 cases (10%) the route of exposure was not given in the source from which the Munro et al. data were taken.

In the database of Bernauer et al. (2008) NOEL data were reported from 24 substances given by inhalation and from 57 (fertility) and 62 (developmental toxicity) respectively given by the oral route. In their paper Kroes et al. (2007) stated in the context of cosmetics that the applicability of TTC values derived from oral data to the topical route has to be based on several considerations. For their investigation they used the existing information in the Munro et al. (1996) database on which to reach conclusions.

Recently, Carthew et al. (2009) presented an inhalation toxicity database and proposed values both for local and for systemic TTC based on existing data. Carthew et al. (2009) proposed a systemic TTC of 980 µg/person per day for substances in Cramer Class I and a TTC of 170 µg/person per day for substances in Cramer Class III, as compared to 1800 µg/person per day for substances in Cramer class I and 90 µg/person per day for substances in Cramer Class III for the oral route by Munro et al. (1996). In addition, the RepDose database of Fraunhofer ITEM is now publicly available (http://www.fraunhofer-repdose.de/), giving information on 650 substances, including information on 203 substances for which administration was by the inhalation route (Escher et al., 2010). The number of substances where the substance was given by the inhalation route was reduced to 136 after those with structural alerts for genotoxicity were excluded. From their inhalation database Escher et al. (2010) derived a systemic TTC of 180 µg/person per day for substances in Cramer Class I and a TTC of 4 µg/person/day for substances in Cramer Class III, which is a factor of between 5 and 40 less than the TTC values of Carthew et al. (2009). The authors discuss the discrepancy between their data and that of Carthew et al. (2009) without a convincing explanation. They come to the conclusion that

further refinement concerning the size of the database and of the definition of structural classes is desirable.

4.11.2. Considerations for route-to-route extrapolation

In general, toxicodynamic as well as toxicokinetic aspects have to be considered when planning to apply the TTC concept to routes of exposure other than the oral route for which the existing TTC levels were derived.

4.11.2.1. Toxicodynamic considerations

Concerning the toxicodynamic aspect of route-to-route extrapolation, it should be understood that in most of the databases the TTC values have been derived from endpoints for systemic toxicity. Hence, the existing TTC values do not encompass portal of entry effects, which may be particularly relevant for the inhalation route. Local effects in the respiratory tract are reported for several chemicals. In the upper respiratory tract not only cytotoxic effects have been described which may lead to loss of olfactory function but also development of cancer, e.g. formaldehyde (McGregor et al., 2006) and vinylacetate (ECB, 2008). In the lower respiratory tract, sensitisation of the airways is an important toxic effect. With other chemicals, cytotoxic effects in the cells lining the airways and the alveoli leading to loss of respiratory function and gas exchange have been observed particularly at high exposure levels. Lung cancer can also be a portal of entry effect, e.g. styrene (Csanady et al., 2003). Portal of entry effects may be also important for dermal exposure with respect to skin sensitisation (van Loveren et al., 2008) but it cannot be assessed by route-to-route extrapolation (Merk, 2009). For systemic toxicity, as a general rule it can be assumed that similar results would be expected by another route of exposure than the oral route if the agent is absorbed by the non-oral route to give a similar internal dose. Hence, route-to-route extrapolation can be considered for systemic effects, whereas it is not possible for local effects.

4.11.2.2. Toxicokinetic considerations

Toxicokinetic aspects to be considered relate to the rate and extent of absorption and possible route specific metabolism. Physiological processes by which organic substances cross the gastrointestinal wall are diffusion through the membranes across the cells, uptake mechanisms by specific transporters and paracellular transport. Diffusion is the predominant process. Hence, absorption through the wall of the gastrointestinal tract is determined by physicochemical properties favouring the absorption of hydrophobic molecules and, in case of weak bases or acids, the non-ionised over the ionised species. Transporters have been identified which play a role in uptake of a substance into the cell and, in some cases, for transport out of the cell back into the gut lumen. For the majority of exogenous substances, the relative importance of such transporters has yet to be elucidated.

Compared to the gastrointestinal tract the skin has a small surface area which is available for absorption which might be further reduced by clothing. The pathway from the outer skin layer (stratum corneum) to the circulation comprises several layers of cells and this slows down the rate (velocity) of absorption. Absorption through the cell layer of the epidermis can be characterised as passive diffusion through a lipophilic structure whereas diffusion through the dermis is characterised as diffusion through a watery layer. The epidermis does not contain vasculature and hence absorption into the systemic circulation can only occur from the dermis layer. Besides lipid solubility, characterised by the octanol/water partition coefficient, water solubility and the molecular mass of the substance are influential on the extent of absorption in a complex pattern. Kroes et al. (2007) predicted the maximum flux through the skin, which is a measure of absorption based on log P and water solubility, and confirmed this complex relationship by examples. They proposed to calculate the flux through the skin by using the octanol/water partition coefficient and the saturation solubility in the vehicle (mostly water) to calculate the flux and proposed a default value for the percentage of the dose absorbed per 24 hours (for formula see publication Kroes et al., 2007). They also concluded that the absorption of a substance with a molecular weight above 500D will be less than 10%.

It should however be considered that solvents and surfactants may influence the extent of absorption as well as the concentration and the condition such as covering the dermal application site (occlusion). For cosmetics, specific consideration has to be given to whether the products are rinsed off. Current EU Guidelines propose a default retention factor of 0.01 for shower gels, shampoo, hair conditioner, 0.05 for toothpaste and 0.1 for hair styling products and for mouthwash (SCCP,2006).
In experimental animals, absorption through skin is generally higher than in humans. Further considerations on dermal absorption, in particular, the influence of dilution on the extent of absorption, can be found in an opinion prepared by the Panel on Plant Protection Products and their Residues (EFSA, 2010d).

Absorption following inhalation has to consider the aerodynamic diameter of the studied aerosols/particulates. Substances with an aerodynamic diameter of greater than 10 μm (man) and 4-6 μm (rat) will not reach the alveolar region but will undergo mucociliary clearance and be swallowed thus reaching the systemic circulation by the oral route. Absorption of particulates in the alveolar region depends on solubility. Insoluble particulates will not be absorbed and will accumulate in the alveolar region and may exert local effects. Substances which enter the systemic circulation by absorption through the alveolar membrane will reach the general circulation before passing through the liver.

4.11.3. Route-specific metabolic factors

The gastrointestinal wall is a metabolically competent tissue. Mucosal cells contain enzymes of the cytochrome P-450 family (phase 1) as well as enzymes capable of conjugation reactions (phase 2). The enzyme activity is lower than in the liver with the notable exceptions of sulphation of beta-sympathomimetic drugs (Dollery et al., 1971; Hildebrandt et al., 1994) and oxidation of some biogenic amines, e.g. tyramine.

The metabolising capacity of the skin has been estimated to be only about 2% of that of the liver whereas others have claimed that the capacity is similar to that in the lung (Baron et al., 2008). Esterases may be an exception as a number of esters have been shown to be hydrolysed during penetration through the skin (Boehnlein et al., 1994).

The lung contains enzymes of the cytochrome P450 family and also conjugation enzymes. The amount of the enzymes present is several orders of magnitude lower compared to the liver. However, the enzymes present may be important in forming active metabolites which may lead to the local production of carcinogens or other toxicologically active substances (Pelkonen et al., 2008).

4.11.4. Pre-systemic metabolism (i.e. first-pass metabolism)

Because of the capacity of the liver for metabolising xenobiotics and the anatomical situation, substances entering the body by the oral route may undergo pre-systemic metabolism, the consequences of which may be different depending on the activity of the parent substance as compared to the activity of the metabolite. If the parent substance is the active species then the substance is assumed to be less toxic when administered by the oral route as compared to the non-oral route. If the metabolite is the active species then the toxicity might be more expressed when the substance is administered by the oral route as compared to the non-oral route. In assessing the relative toxicity of the oral and the non-oral route, it is important to consider (1) the target organ for toxicity, and (2) the relevant toxicokinetic metric, i.e. the amount of the substance or its metabolite in the systemic circulation versus the absolute concentration of the substance or its metabolite in the target organ.

4.11.5. Criteria for route-to-route extrapolation

The following general criteria have been proposed by Pepelko (1987) as a basis for deciding whether route-to-route extrapolation can be performed. These criteria were taken up by the UK IGHRC (Interdepartmental Group of Health Risk from Chemicals) in their guidelines (IGHRC, 2006).

- Absorption is the same between routes, or the difference is known and can be quantified.
- The critical target tissue is not at the portal of entry of the compound.
- There is no significant metabolism of the chemical by oral, gut or skin enzymes or in pulmonary macrophages, or transformation by other processes in the gut or lung.
- First pass effects are minimal.
- The chemical is relatively soluble in body fluids.

However, it is not straightforward to apply the above mentioned criteria as a tool for adapting the TTC concept to routes of exposure other than the oral route since for most substances to be evaluated by the TTC approach the relevant information would not be available.

4.11.6. Default approaches

In the absence of data on the extent of absorption, an extrapolation can be based on the physicochemical properties of the substance taking into account results from analysing existing data or a read-across approach from structurally analogous substances. In the Munro et al. (1996) database 170 (27.7 %) of the substances were given by gavage and 36 (5.9%) by drinking water as opposed to 344 cases (56.1%) where the substance was given by diet. Administration of the dose by gavage results in high maximum concentrations in the blood whereas it is expected that dietary administration will lead to more moderate maximum concentrations. Administration by drinking water will result in maximum concentrations in between. Hence, the Munro et al. (1996) database contains representation of different modes of application with high and moderate maximum concentrations. It can be concluded that this aspect is well covered.

4.11.7. Extrapolation route oral to dermal

In order to conduct oral to dermal extrapolation, an assumption has to be made that absorption is the same between routes, or the difference is known and can be quantified. An approach that could be adopted would be to follow the recent recommendations from EFSA concerning plant protection products (PPPs).

In the EU, for establishment of dermal absorption values for PPPs, in the absence of valid measured data, a default value of 100% is applied. A lower default value of 10% is applied if the active substance has a molecular weight of above 500 and a log Pow value of either below -1 or above 4 (EC, 2004). EFSA has recently proposed some further refinements of these basic default values for application to PPPs (EFSA 2010).

Kroes at el. (2007) explored the possibility to use the Munro et al. (1996) database as a basis for deriving dermal TTC values. In their view, the only situation where the oral TTC would underestimate the dermal TTC (after correction has been made for absorption through skin as compared to the gut wall) would be if the substance exhibited high pre-systemic metabolism. They calculated the situation for a substance with a pre-systemic metabolism of 50% and came to the conclusion that this case would be covered by the conservative assessment/extrapolation factors.

In addition, they analysed the database of Munro et al. (1996) and came to the conclusion that that the majority of Cramer class III compounds do not undergo pre-systemic detoxication after oral dosing, but that many would show higher toxicity after oral dosing because hepatic first-pass metabolism results in the generation of a toxic metabolite. Hence, they feel that an additional factor would not be necessary and the topic seems not to be relevant.

4.11.8. Extrapolation route oral to inhalation

For extrapolation from the oral to the inhalation route the situation is at present not simple. As an alternative, Carthew et al. (2009) proposed TTC values for local and for systemic effects based on

existing inhalation data. They specifically addressed substances likely to be present in consumer products. The data they used excluded substances with certain properties, such as genotoxic carcinogens *in vivo* mutagens (presumed carcinogens), potential respiratory sensitisers, potential irritants (strong acids or bases), and pharmacologically active substances, together with certain other groups of substances, such as heavy metals (neurotoxic), dioxins and PCBs (accumulative and biopersistent), organophosphates (neurotoxic), and polymers (require substance-specific data).

An extrapolation approach has been taken by the IGHRC (2006) where specific extrapolation factors have been derived (see Appendix F). However it should be noted for substances undergoing TTC evaluations, that there would normally be very few data available for refining the assumptions on bioavailability.

4.11.9. EFSA considerations on route-to-route extrapolation

Proposed TTC values are based on the existing Munro et al. (1996) database. The NOELs represent systemic toxicity endpoints and the route of administration in the studies was the oral route. When considering whether to use the TTC values derived from this database of oral studies for substances and situations with a non-oral route of exposure it should be borne in mind that portal of entry effects are not covered which may be of relevance. Concerning systemic effects, default factors can be used to account for the route-specific extent of absorption. Extrapolation approaches have been proposed by the IGHRC (2006) and also by Kroes et al. (2007) for extrapolating from the oral to the dermal route.

The Scientific Committee recognises that the use of the oral TTC values for extrapolating to the dermal route of exposure would require knowledge of the oral bioavailability of the substances of the Munro database. This information is not available. The oral TTC values could be considered for use if it was known from experimental data that dermal absorption was low (e.g. 10% or lower) because there would be reasonable confidence that these TTC values would not underestimate the risk from the dermal route of exposure. However, it would be preferable to develop a specific dermal toxicity database to establish dermal TTC values.

If the route-to-route extrapolation is oral to inhalation, given the most recent findings it seems not advisable to base the TTC on the Munro et al. (1996) data and perform route-to-route extrapolation. The proposals of Carthew et al. (2009), who used inhalation data to derive TTC values, are based on a small number of chemicals (92). Because of the long list of exclusion criteria, the proposed TTC values can only be applied if several properties of the substance in question are known from experimental data. The publication of Escher et al. (2010) is based on 136 chemicals. They derived TTC values which are one order of magnitude lower than the Munro et al. (1996) TTC values with 180 µg/person per day for Cramer Class I substances and 4 µg/person per day for Cramer Class III substances. The TTC values derived by Escher et al. (2010) are recognised to be conservative because they include consideration of toxicity resulting from local effects on respiratory tract. However, further extension of this database would be desirable before establishing TTC values for inhalation.

4.12. Potential for application of the TTC concept in the different EFSA Panels

4.12.1. ANS Panel

The Panel on Food Additives and Nutrient Sources has responsibillity for evaluating additives in human food and the safety of substances used in nutrient sources. For these substances usually toxicological data on the main components will be available. The TTC approach could be relevant for evaluating impurities and breakdown/reaction products in food additives and nutrient sources.

4.12.2. CEF Panel

The Panel on Food Contact Material, Enzymes and Flavourings has responsibility for evaluation of several different types of substances, notably food contact materials and flavouring substances. For flavourings, the TTC approach is already used by the Panel (see 2.5. and Appendix B) and it would seem logical that the same approach might also be used for food contact materials, where exposures can be low. Currently, food contact materials are evaluated through a tiered approach that was adopted by the Scientific Committee on Food at the end of the 1980s and which continues to be used by EFSA. Under this tiered approach, different toxicological datasets are requested according to the migration of the substance in food simulants, with more testing required for higher migration. The TTC approach could be useful for substances with low-level migration from food contact materials. Migration of impurities and side-products resulting from the manufacture of the final article also may need to be considered and application of the TTC approach to such situations could be helpful.

The discrepancy between the method used for the safety evaluation of flavourings and that for food contact materials is currently under discussion in the Panel and special attention will be paid to the recommendations in this opinion of the Scientific Committee.

4.12.3. CONTAM Panel

The Panel on Contaminants in the food chain deals primarily with contaminants that are often data rich and in many cases do anyway not qualify for the TTC approach (e.g. dioxins, aflatoxins, heavy metals). Examples of areas in which application of the TTC concept could be envisaged are trace contaminants in (bottled) water and trace contaminants resulting from previous cargoes.

4.12.4. FEEDAP Panel

The Panel on Feed Additives deals with additives and products or substances used in animal feed. The Panel is responsible for the assessment of the safety for target species, i.e. animal species for which the additive is intended to be used, the safety for consumers (integrating toxicology and carry-over to consumers via edible tissues/products), safety for users (taking into account inhalation and dermal exposure), safety for the environment and efficacy, the latter two items being outside the remit of this opinion.

Within this general framework, the assessment may present specific features depending on the type of additive. For instance, no carry-over studies are normally foreseen for the wide group of micro-organisms and enzymes. As for established nutrients (vitamins, trace elements) pivotal elements for risk assessment are the carry over into edible tissues/products and additional exposure of animals and consumers, compared to existing background.

There would be possibilities for the application of the TTC approach to consumers safety within the FEEDAP Panel's remit, of activity. Since exposure of consumers is related to the metabolism of target species, comparative pharmacokinetic studies should show whether the same metabolites are:

- covered by testing in toxicological studies on laboratory animals, and
- present as residues in target farm animals.

On occasion, one or more metabolite(s) are encountered in target farm animals that are not formed in laboratory animals and represent at least 10% of the total residues (metabolites representing less than 10% of the total residue are normally not considered). Such metabolites are chemically identified, but not toxicologically characterised. Currently, toxicological testing for such metabolites is required on a case-by-case basis and the TTC approach could be an appropriate tool in order to address whether, and to what extent, testing should be performed.

4.12.5. PPR Panel

The Panel on Plant Protection Products is not directly involved in the approval process for plant protection products. Rather, it is consulted when there is a toxicological issue that cannot be resolved

during the normal approvals process. In addition, the Panel is increasingly becoming involved in the preparation of guidance documents.

In addressing a specific toxicological issue, the Panel would adopt a chemical-specific approach. In general, for active substances, there are specified data requirements, and hence the need for the TTC approach would not be an issue. It could be argued that for plant protection products resulting in minimal residues on crops, the TTC approach might be relevant, but to date this has not been foreseen in the legislation.

An area where the TTC approach is being actively considered by the PPR Panel is that of the toxicological relevance of plant metabolites and degradates of pesticide active substances. The broader issue of how to assess such substances will be the subject of a forthcoming opinion of the Panel. As part of this activity, there has been an assessment of the applicability of the TTC approach to such an evaluation, under Article 36. The report of this activity is available on the EFSA website EFSA, 2010a). The PPR Panel is of the view that the TTC has potential application in the assessment of the toxicological relevance of plant metabolites and degradates of pesticide active substances. Metabolites either predicted by software tools or identified analytically would be assessed for structural alerts for genotoxicity, using appropriate software, and for the respective Cramer class, which can also be achieved using a freely available software package. Predicted dietary exposure to the metabolite or degradate would be compared with the appropriate TTC value. A number of aspects of this strategy are still under discussion and will not be finalised until the Panel adopts its opinion by the end of 2011.

CONCLUSIONS AND RECOMMENDATIONS

The Scientific Committee has considered a number of published analyses and conducted some analyses itself of the data originally used to establish human exposure threshold values (TTC values), i.e. the Munro et al. (1996) database. The Scientific Committee has also conducted analyses of data from studies that are not necessarily included in the original Munro et al. database, using EFSA's databases on pesticides and an EU database of substances classified for reproductive toxicity. EFSA also commissioned a project from a contractor to examine the databases underpinning the TTC approach, using *in silico* chemoinformatic methods to assess the representativeness of the databases and the opportunities for refining the basis for grouping chemicals. Further analyses of oral toxicity data and TTC values have also been conducted and published by others using independent databases. The outcomes of these analyses have been discussed and the Committee's conclusions and recommendations follow.

w. The TTC approach is a useful screening tool for both qualitative risk assessment and priority setting that enables efficient use of available resources and potential reductions in animal testing. The TTC approach is mostly applicable to substances for which the chemical structure is known but there are few or no relevant toxicity data. It would not normally be applied when there is a legislative requirement for submission of toxicity data.

x. For application of the TTC approach it is essential to have suitably conservative exposure assessments, which take account of high exposure scenarios. It requires information on known or predicted human exposures, for which there is confidence that they are not an underestimate. The EFSA Panels already have in place suitable exposure assessment methodologies for predicting or estimating average and high exposures in relevant sub-populations, and the EFSA Comprehensive European Food Consumption Database is expanding..

y. The classification of chemicals according to chemical structure is an important component of the current TTC approach. The classification scheme most widely used is that described by Cramer et al. (1978). The Scientific Committee is mindful that this scheme is based on the metabolic and toxicological information available at that time. With advances in knowledge over the last three decades, revision and refinement of the scheme would be timely. Nevertheless, the Scientific Committee's analyses, together with those of the EFSA-commissioned project and several other published studies (referenced elsewhere in this report) have demonstrated that the application of the Cramer classification scheme in the TTC approach is conservative and therefore protective of human health. In this respect, the Cramer scheme can be regarded as fit-for-purpose in the context of regulatory advice.

z. The Scientific Committee notes that the TTC value for Cramer Class II substances derived by Munro et al. in 1996 was on the basis of toxicological data on very few substances. Databases compiled subsequently have similarly found few chemicals classifiable as Cramer class II, apart from flavouring substances. The Committee considers that the TTC value for Cramer Class II is not well supported by the presently available databases and therefore concludes that consideration should be given to treating substances that would be classified in Cramer Class II under the Cramer decision tree as if they were Cramer Class III substances.

aa. The Committee's analysis of the lowest 10^{th} percentiles of the NOELs in the database of Munro et al. (1996) for substances in Cramer Class I and Class III, and confirmation by others of similar NOELs using a different dataset (Escher and Mangelsdorf, 2009) demonstrate that the respective TTC values of 1800 and 90 µg/person per day derived by Munro et al. are sufficiently robust and conservative to be used.

bb. Following the Scientific Committee's analysis of NOELs for organophosphate and carbamate substances, the TTC value of 18 µg/person per day, first proposed by Kroes et al. (2004), is considered sufficiently robust and conservative to cover the anti-cholinesterase activity of OPs and carbamates. Removing such substances from Cramer Class III (which are the most potent substances in that class) might be considered to have an impact on the existing TTC value for Cramer Class III. However, pending any future revision of the TTC approach, the Committee concludes that it would be prudent to maintain the value for Cramer Class III at 90 µg/person per day.

cc. The Scientific Committee considers that additions to or subdivisions of existing Cramer Classes are likely to detract from the advantageous features of the current TTC scheme, that is, its ease of use, maintaining consistency in application of the approach, and its in-built conservatism.

dd. Following the Scientific Committee's analysis of NOELs for reproductive and developmental toxicity for substances classified as such under EU legislation, the TTC values for Cramer Classes I and III are considered sufficiently protective for adverse effects on reproduction or development.

ee. Substances with endocrine-related toxicity have been assessed extensively and comprehensively in hazard and risk assessment procedures that were in place when the Munro et al. (1996) database was compiled. They encompass a wide range of endocrine-mediated adverse effects including reproductive and developmental toxicity as well as, for example, thyroid and adrenal toxicity. In addition, the Scientific Committee's analysis of the more recent data on reproductive and developmental toxicity, based on studies using existing globally harmonised test protocols, showed that the TTC values are adequately protective. The analysis of the substances in the lowest 10^{th} percentile of the Cramer Class III group in the Munro et al. database also indicated that adverse effects on reproduction and development were likely to be covered by the existing TTC value. It is concluded that adverse effects of endocrine-related toxicity are adequately covered by the existing TTC values.

ff. For substances with a structural alert for genotoxicity, the TTC value of 0.15 µg/person per day was derived by Kroes et al, 2004. This is sufficiently robust and conservative to be used in EFSA's work, provided the structures already designated as high potency carcinogens are excluded from the TTC approach. The Scientific Committee is aware that further substances have been added to the CPDB since this value was derived. However, because a large number of substances was already in the CPDB, the Committee does not consider that the TTC value for substances with structural alert for genotoxicity would change appreciably.

gg. The Scientific Committee has considered the possibility that a genotoxic metabolite could be produced from a parent substance without any structural alert for genotoxicity. If such metabolites were to be predicted , then the TTC value of 0.15 µg/person per day should be applied. The Scientific Committee recognises that there is no general agreement at present on how such metabolites could be predicted.

hh. The original FDA Threshold of Regulation value of 1.5 µg/person per day is of historical importance, but has little practical application in the overall TTC approach. This is because substances without structural alerts for genotoxicity can proceed down the TTC decision tree to be considered in relation to the higher TTC values for Cramer Classes I and III (unless they are OPs or carbamates). Non-genotoxic carcinogens are considered to be thresholded and, in general, NOELs for these are in the same range or higher than NOELs for others non-cancer endpoints. The Cramer Class TTC values are therefore also applicable to substances for which it is not known whether they may be non-genotoxic carcinogens.

ii. The Scientific Committee also notes that the work of the EFSA-commissioned project demonstrated that the range of structures in the two main datasets, which underpin the human exposure threshold values, are broadly representative of the world of chemicals, in terms of chemical space, as described by molecular descriptors encompassing both structural features and physicochemical properties. This provides further confidence in the general utility of the TTC approach.

jj. A number of proposals have been put forward for adjusting TTC values for shorter than chronic durations of exposure. The Scientific Committee is not confident about the general applicability of these proposals and also notes that the current TTC values are derived from databases that do not address effects from acute exposure. Instead, such situations should be addressed case by case, for example by considering the margin between the unadjusted TTC value and the estimated dietary intake.

kk. For application of the TTC approach to the whole population including infants and children, all TTC values should be converted to corresponding values that take into account body weight (see Figure 2).

ll. The Scientific Committee has also considered whether the TTC approach could be applied to young infants under the age of 6 months, in whom metabolic and elimination processes are not yet mature. If the estimated exposure is in the range of the TTC value, careful consideration would need to be given as to whether the outcome of the TTC approach can be used.

mm. The Scientific Committee has considered whether the TTC approach could be applied to routes of exposure other than oral. For the oral to dermal route, default procedures are available that could be used to predict systemic exposures. However, it should be borne in mind that portal of entry effects would not be covered and these may be of relevance. It would therefore be preferable to develop TTC values from a dermal toxicity database. For the inhalation route, it would also be desirable to further extend the toxicity database that has been compiled by Escher et al (2010) before recommending TTC values for inhalation.

nn. The Scientific Committee considered whether routinely undertaking metabolic prediction would be helpful for application of the TTC approach other than for prediction of genotoxicity. As the Cramer decision tree and the databases used to derive the TTC values for non-cancer endpoints reflect at least in part the toxicity of metabolites formed in the test species, the Scientific Committee concluded that it is not essential to undertake such metabolic prediction. However, there may be situations where this would be helpful, e.g. in cases where metabolic data on closely structurally-related substances are available (such as in the case of flavourings).

oo. The Scientific Committee considered both previously proposed exclusions and additional exclusions that might be necessary and concludes that the TTC approach should not be used for the following (categories of) substances:

- High potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds).
- Inorganic substances
- Metals
- Proteins
- Substances that are known or predicted to bioaccumulate .
- Substances with structures that are not adequately represented in the original databases from which the TTC values have been derived, e.g. nanomaterials and radioactive substances.
- Substances likely to have the potential for local effects on the gastro-intestinal tract

pp. Areas within EFSA's remit in which the TTC approach may be useful include, but are not necessarily limited to, low-level exposures to:

- Substances in food contact materials and their impurities and breakdown/reaction products
- Plant metabolites and degradates of pesticide active substances
- Metabolites of feed additives formed in target species that are not covered by tests in laboratory species
- Technological feed additives
- Flavouring substances in feed
- Trace contaminants in food and feed, including bottled water
- Impurities and breakdown/reaction products in food additives

qq. The Scientific Committee recognises that when the different EFSA panels apply the TTC approach to their respective areas, specific considerations may be needed.

rr. Wider use of the TTC approach in EFSA's work would contribute to reducing unecessary animal use in toxicity testing.

From the above conclusions, a generic scheme for the application of the TTC approach has been developed and it is shown in figure 2 below:

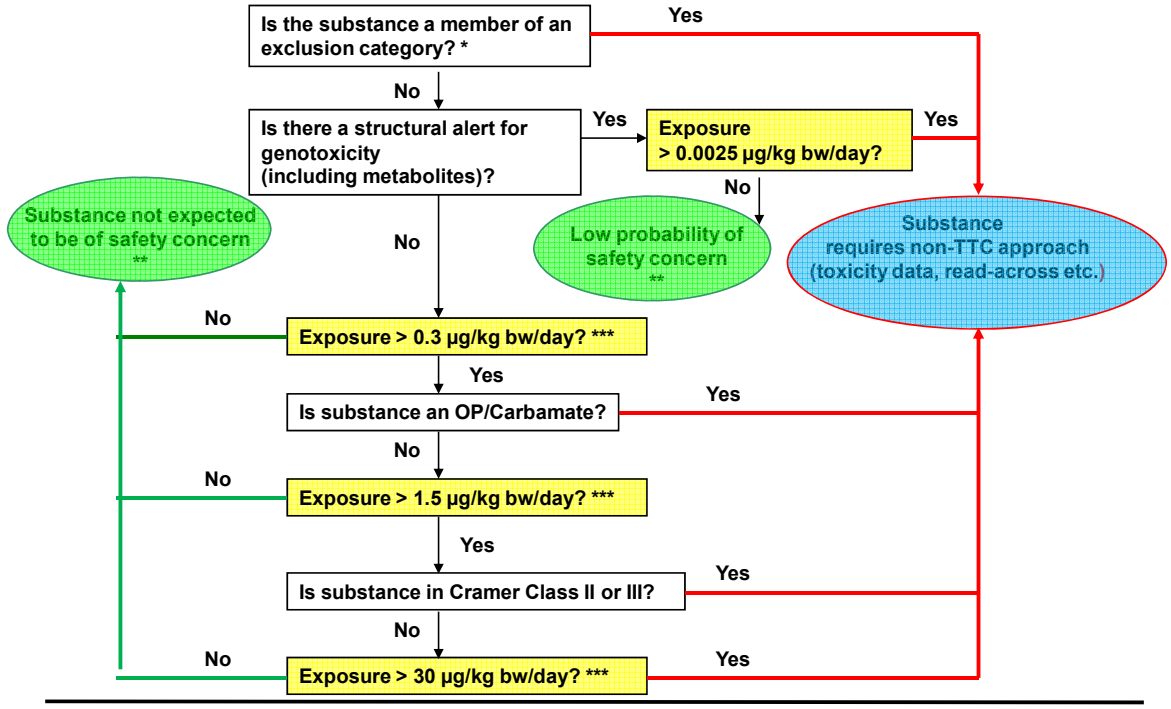

Figure 2: Generic scheme for the application of the TTC approach

Recommendations for future work

a. In the short-term, it is recommended that the Cramer scheme should be rewritten, making it more transparent and easier to understand. Such an exercise should take into account the rules that work well, but also make changes (rule additions, rule deletions, revisions in rule scope and ordering, where necessary).

b. In the longer term it may be desirable to develop classification schemes that are more discriminating between different toxicity groups.

c. In view of the current reservations about the use of Cramer Class II, it would be desirable to investigate the number of classes that would be necessary for the practical application of TTC approach, which may differ in different areas.

d. For shorter than lifetime exposure, particularly acute exposure, further work is needed to identify appropriate TTC values.

e. Further work is needed on improving the accuracy and/or the efficiency of *in silico* tools for predicting genotoxic potential for the substance itself and its metabolites; work on prediction of metabolite formation will also be necessary.

f. If further substances are added to the databases that currently underpin the TTC approach, it would be desirable to express all toxicity values as BMDLs rather that NOELs and express potency in terms of molar equivalents rather than mass per body weight.

g. Normally for risk assessment, all sources of exposure for similarly acting substances should be taken into account, including non-food sources of exposure. In addition, for any one substance, all routes of exposure should be taken into account. The Scientific Committee recognises that at present this is difficult, especially in view of the current limitations on route-to-route extrapolation. The Scientific Committee recommends that work be done to further explore this issue in the context of the TTC approach, which may require the further development of dermal and inhalation toxicity databases.

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APPENDICES

APPENDIX A

Historical development of the TTC concept

Frawley (1967) was the first to propose the idea that there was a general threshold level for chemicals in the diet, below which the risk to human health would be negligible. His proposal was made in the context of safety assessment of substances used in food packaging materials, many of which were then untested and of unknown toxicity. He analysed a data set of 2-year, chronic toxicity studies in animals on 220 different chemicals given via the diet and identified the doses below which no toxicological effects were observed. The substances involved were food additives, industrial chemicals, chemicals found in consumer products including cosmetics, chemicals used in food packaging materials, pesticides and heavy metals. The studies represented about 90% of all the available chronic toxicity data at that time. From this analysis, Frawley selected a concentration of 10 mg/kg of diet, since very few chemicals (19 out of 220) and only those of a type not likely to be used in food packaging (i.e. heavy metals and pesticides) showed toxicity below this level. This concentration was divided by 100 to provide a margin of safety, giving a figure of 0.1 mg/kg of human diet. This was the dietary concentration for any substance migrating from food packaging materials which he considered could be consumed without risk to human health.

The issue of toxicologically insignificant levels of chemicals in food was further considered in guidelines issued in 1969 by the Food Protection Committee of the US National Academy of Sciences (NRC, 1969). The Committee noted that certain categories of chemical could have deleterious effects at low doses, namely certain impurities or contaminants of natural origin (such as aflatoxin and botulinus toxin), certain essential nutrients and hormones, certain heavy metals and their compounds, and certain organic compounds employed for their biological activity (included in the latter three categories were pesticides, pharmaceuticals and antipersonnel agents that may have biological activity at levels as low as 0.1 ppm). Aside from these, they noted that the analysis of Frawley (1967) had shown that no other substance had produced toxic reactions in experimental animals below a dietary concentration of 40 mg/kg. The Committee concluded that, with the exception of the potentially more toxic types of chemical mentioned above, a concentration of 0.1 ppm (0.1 mg/kg) of a chemical in the human diet could be presumed to be toxicologically insignificant. For substances with simple structures and known purity, that would be readily metabolised, and which belonged to a group of substances that were known or presumed to be of low toxicity, the Committee concluded that a higher concentration of 1 ppm (1 mg/kg) could be presumed to be toxicologically insignificant.

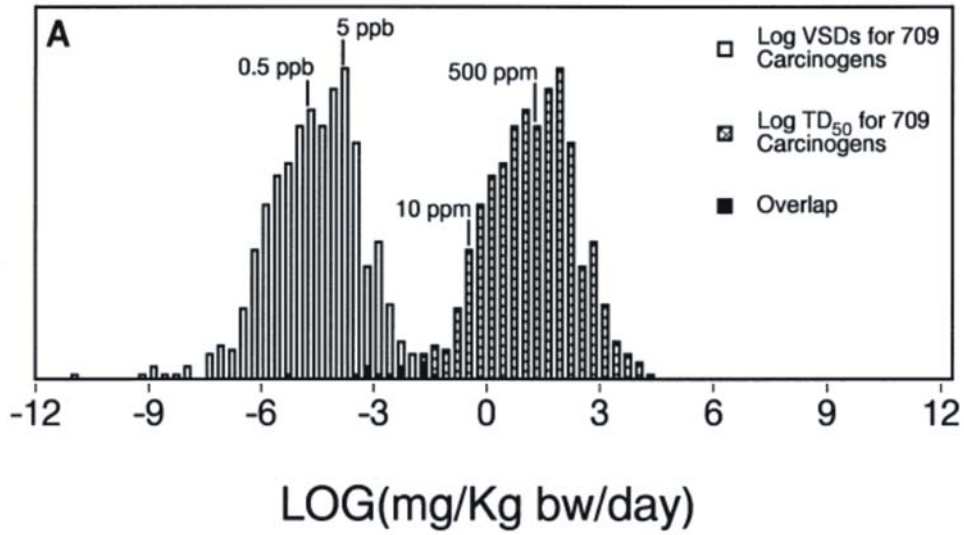
Development of human exposure threshold values for the endpoint of cancer

US FDA 'Threshold of Regulation'

The first regulatory body to formally derive a threshold value related to the toxicological endpoint of cancer was the US Food and Drug Administration (FDA). The FDA was concerned that the agency should focus its limited resources for risk assessment of food contact materials on issues of tangible concern rather than trivial ones. The Threshold of Regulation (TOR) policy was developed over 10 years (Rulis, 1986, 1989, 1992), during which the agency examined relevant scientific data that would enable them to set a threshold, intended to protect against all types of toxicity including carcinogenicity, for application in food packaging regulation. In 1995, the FDA adopted the TOR policy for substances present in food contact materials (FDA, 1995). Such substances are also termed indirect food additives in the USA and are regulated as such. The TOR policy contains elements of both scientific and risk management judgments. The term "Threshold of Regulation" is used, rather than "threshold of toxicological concern", but the science underlying the policy is analogous to the TTC concept. A threshold value was derived from cancer data since this was considered to be the

toxicological endpoint most likely to be triggered by exposure to low doses of chemicals. The approach was based on an analysis by Gold et al. (1984) of nearly 500 chemical carcinogens, later expanded to over 700 (Gold and Zeiger, 1997), that had been tested in animals using lifetime exposures, known as the carcinogenic potency database (CPDB). In this database, the potency of each chemical is expressed as a TD_{50} . The TD_{50} is defined as the daily dose-rate in mg/kg body weight per day for life to induce tumors in half of the test animals that would have remained tumor-free at zero dose. In cases where there are multiple data, the TD_{50} is derived from the most sensitive species, strain and sex. The TD_{50}s were plotted as a distribution (see Figure 1). Linear extrapolation was then used to derive an estimate of the dietary concentration of most carcinogens which would give rise to less than a one in a million lifetime risk of cancer (1 x 10^{-6}, termed a 'virtually safe dose'), assuming that the risks in animals are representative of those in humans, and these were also plotted as a distribution (see Figure 1). From the distribution of virtually safe doses, a dietary concentration of 0.5 ppb was selected as the value to use for the TOR.

Figure 1. Distribution of TD50s for chemical carcinogens and extrapolation to 1 in a million risk



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From the dietary concentration of 0.5 ppb, a human daily exposure level of 1.5 µg/person was derived, assuming that an adult may consume 1500 g of food and 1500 g of fluids daily and that the substance is distributed throughout the total diet. If dietary exposure to an individual substance was below the threshold, the agency considered that consumers would be protected "*with reasonable certainty of no harm*", even if that substance was later shown to be a carcinogen. With respect to other non-cancer effects, the agency noted as follows: "*A 0.5 ppb threshold is 2000 times lower than the dietary concentration at which the vast majority of studied compounds are likely to cause non-carcinogenic toxic effects and 200 times lower than the chronic exposure level at which potent pesticides induce toxic effects*" (FDA, 1993). Application of the TOR policy in the USA means that substances in food-contact articles that are present in the diet at concentrations at or below 0.5 ppb are exempted from regulation as a food additive and no toxicity testing on them is required. Substances that have been shown to be carcinogens in humans or animals, or, on the basis of their structure, are suspected of being carcinogens are excluded from consideration under the TOR.

Following the adoption of the TOR policy in the USA, subsequent work by the FDA on carcinogenic potency provided support for the use of thresholds for human dietary exposure that were higher than 1.5 µg/person per day. Using the then expanded carcinogenic potency database of 709 chemicals (Gold and Zeiger, 1997), together with short-term toxicity data, results of genotoxicity testing and structural alerts, Cheeseman et al. (1999) identified potent and non-potent subsets. This work

confirmed the validity of 1.5 µg/person per day as an appropriate threshold for most carcinogens, but went on to propose a tiered threshold of regulation. Examination of the expanded database led them to conclude that a threshold of 4 - 5 µg/kg of diet could be appropriate for substances without structural alerts and even for substances with structural alerts if they were negative in tests for genotoxicity. If substances had no structural alerts, were negative in tests for genotoxicity, and had acute toxicity (LD_{50}) above 1000 mg/kg bw, they proposed that a threshold of 10 - 15 µg/kg of diet could be used. To date, these proposals for a tiered approach within the TOR have not been adopted by the FDA.

Proposal of a threshold for substances with a structural alert for genotoxicity

As can be seen from Figure 1, approximately one-third of carcinogens have TD_{50}s that result in extrapolated virtually safe doses below 0.5 ppb. Kroes et al. (2004) have therefore refined the threshold for the endpoint of cancer by deriving a lower value for substances containing a structural alert for potential genotoxicity. They used the same modeling approach as previously used by the FDA (i.e. linear extrapolation from the TD_{50}), to calculate exposures estimated to increase the lifetime risk of cancer by 1 in a million (1×10^{-6} risk). Analysing of a database of 730 substances (709 substances extracted by Cheeseman et al. (1999) from the Gold CPDB (Gold and Zeiger, 1997) plus additional substances), they focused on identifying the structural alerts that would give the highest calculated risks if present at very low concentrations in the diet. In order to identify the structural groups of most concern, the scheme of structural alerts proposed by Ashby and Tennant (1991) and by Cheeseman et al. (1999) was examined. The differences between the different structural alerts was most apparent in the data for the fraction of substances within each group giving an estimated upper bound risk of cancer of greater than 1×10^{-6} when present in the diet at a concentration of 0.15 µg/person per day. This value was therefore selected as the generic TTC for substances with a structural alert for genotoxicity, and is 10-fold lower than the US TOR of 1.5 µg/person per day. The substances for which the risk was greater than 1×10^{-6} at an exposure of 0.15 µg/person per day were further examined (see 2.2.3 below).

In the mean time, the Gold database has been updated and a supplement was added in 2007[20]. The database contains now more than the 730 substances used by Kroes et al. (2004) to derive the TTC of 0.15 ug/person and day. However, because of the large number of substances already in the earlier database, the Scientific Committee considers that the distribution of 1×10^{-6} risk levels derived by linearised low-dose extrapolation for these 730 carcinogens would not be expected to change substantially if the new substances were to be included in the analysis, provided structural groups of high potency carcinogens as defined by Kroes et al. (2004) were excluded.

Exclusion of very potent carcinogens

During their assessment of variations in carcinogenic potency, Cheeseman et al. (1999) identified some groups of substances in which a high percentage of those tested had virtually safe doses below 0.5 ppb. They therefore proposed that such groups should be excluded from exemption under the TOR. These were (1) substances with *N*-nitroso or benzidine-like structural alerts, even if they were negative in the Ames assay, and (2) hydrazines, triazenes, azides, azo and azoxy substances, and substances with strained heteronuclear rings, that test positive in the Ames assay.

The issue of very potent carcinogens was further explored by Kroes et al. (2004). They identified 3 structural groups of genotoxic carcinogens — aflatoxin-like compounds, N-nitroso-compounds and azoxy-compounds — which are of such high potency that if a TTC were to be established to cover all these it would need to be set at a much lower dietary concentration than the generic TTC for other structural groups of genotoxic carcinogens. They also identified some unusual high-potency non-genotoxic carcinogens — TCDD and steroids. They concluded that establishing a TTC that would cover these high-potency structural groups, termed the "cohort of concern", would not be appropriate.

[20] http://potency.berkeley.edu/database.html, accessed on 17.3.2009

They therefore concluded that compounds with these structural alerts for high potency require compound-specific toxicity data and should be excluded from the TTC approach.

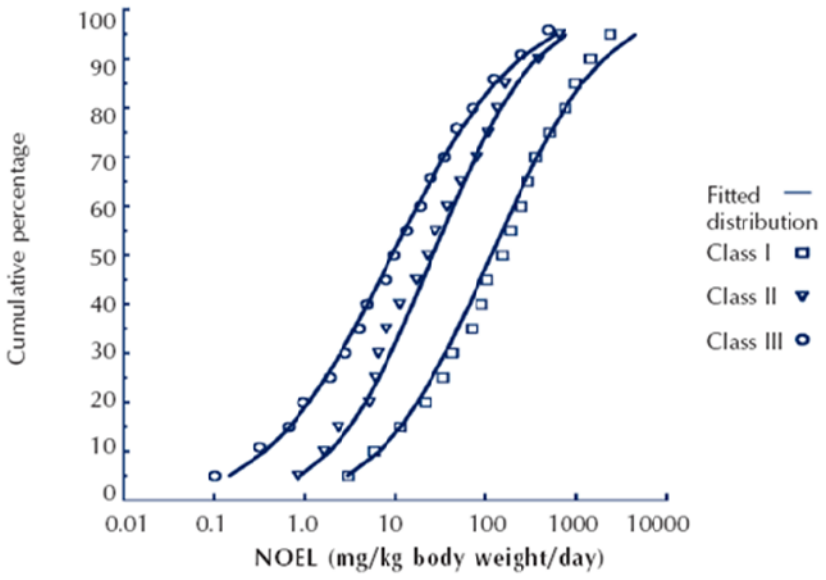
Development of human exposure threshold values for non-cancer endpoints

The work of Munro and colleagues

Around the same time as the FDA was developing the TOR policy, Munro and colleagues were developing the TTC concept (Munro 1990, 1996; Munro et al., 1996, 1998, 1999). In a key paper (Munro et al., 1996) they proposed the use of generic thresholds for acceptable human exposures based on an exploration of the relationship between chemical structures and toxicity. They compiled a large reference database (hereinafter referred to as the Munro et al. database) consisting of 613 chemicals for which toxicity data were available on a variety of non-cancer endpoints from subchronic, chronic, reproductive and developmental toxicity studies. Over 2900 no-observed-effect levels (NOELs) were available from these studies. The chemicals in the Munro et al. (1996) database were divided into three structural classes, based on a "decision tree" developed earlier by Cramer et al. (1978). The criteria for the three structural classes are shown in section 3.1 of the main opinion.

The Cramer et al. (1978) decision tree is based on a series of 33 questions relating mostly to chemical structure, but natural occurrence in food and in the body are also taken into consideration. The logic of the sequential questions was based on the then available knowledge on toxicity and on how chemical structures were metabolised in mammalian metabolic pathways. Cramer et al. (1978) predicted that the majority of substances would fall into either Class I (likely to be of low oral toxicity) or Class III (no strong presumptions of safety or suggestive of significant toxicity), rather than Class II (intermediate), and that is indeed borne out by the Munro et al. database and by subsequent experience with the TTC approach. Cramer et al. (1978) tested the validity of their decision tree by classifying 81 chemicals (used as food additives, drugs, industrial chemical or pesticides), on which toxicity data from short-term or chronic studies were available, into the three structural classes and tabulating the NOELs. There was overlap in the range of magnitudes of the NOELs between the three structural classes, but it was clear that the NOELs of Class I substances were generally higher than those of Class III, with those of Class II being in between.
Munro et al. (1996) followed the approach of Cramer et al. (1978), classifying each of the 613 substances in their database into its Cramer structural class. There were 137 substances classified in Class I, 28 in Class II and 448 in Class III. They then identified the lowest NOEL for each substance from the available toxicity data and plotted the magnitude of the NOELs for each class in cumulative distributions (see Figure 2).

From each of the three lognormal distributions, they estimated the 5^{th} percentile of the distributions of NOELs. To derive "human exposure thresholds" for each structural class, the 5^{th} percentile values were multiplied by 60 (assuming an individual weighs 60kg) and then divided by a factor of 100 to ensure a margin of safety. The three "human exposure thresholds" obtained, in mg/person per day, are shown in Table 1. These human exposure thresholds are also referred to as TTCs.



Cumulative distribution of the most conservative NOELs for substances in the reference database grouped into Cramer et al. (1978) structural classes I, II and III, fitted lognormal distribution (Copyright with permission from Elsevier).

Table 1: Derivation of human exposure thresholds from toxicity data

Structural class	Fifth percentile NOEL (mg/kg bw per day)	Human exposure threshold (µg/person per day)*
I	3.0	1800
II	0.91	540
III	0.15	90

* The human exposure threshold was calculated by multiplying the 5th percentile NOEL by 60 (assuming an individual weighs 60 kg) and dividing by a safety factor of 100.

Munro and colleagues emphasised that the human exposure thresholds are intended to apply only to structurally defined chemicals for which there is no evidence of genotoxic carcinogenicity and no structural alerts for genotoxicity. According to this scheme, a threshold can be selected for a chemical of known structure but unknown toxicity; if human exposure to a chemical is below the relevant threshold of concern for its structural class, Munro and colleagues considered that "the substance can be judged, with reasonable confidence, to present a low probability of risk" (Munro et al., 1996).

Cheeseman et al. (1999) also examined the underlying premise of the US TOR policy, that by using a threshold that protects against carcinogenic effects, it would also protect against other toxic effects. They analysed information from the Registry of Toxic Effects of Chemical Substances (RTECS) on 3306 substances for which there were oral reproductive toxicity data and 2542 substances for which there were data from other repeat-dose toxicity tests. For each substance, they searched for the lowest dose at which a toxic effect was seen and divided this lowest low effect level (LLEL) by an uncertainty factor of 1000 to derive a range of "pseudo-acceptable daily intakes" (PADIs). The most likely (median) value for the Pseudo Acceptable Daily Intake for the reproductive toxins was 10 ppm (10 mg/kg diet), which was 8300-fold above 1.2 ppb, corresponding to the median value for the one in a million risk levels for carcinogens, estimated from the carcinogenic potency database. These results supported the presumption that a 'virtually safe dose' based on carcinogenicity data would protect against other non-cancer, toxic effects. Comparison of the Pseudo Acceptable Daily Intakes

(LLEL ÷ 1000) for non-cancer effects with the "ADIs" from the Munro et al. (1996) database (NOELs ÷ 100) showed that the Pseudo Acceptable Daily Intakes were one order of magnitude more conservative than the "ADIs", reflecting the 10-fold difference in the uncertainty factor applied.

Exclusion of certain groups of substances from the TTC approach

In addition to recommendations to exclude substances with structural alerts for high potency carcinogenicity (see 2.3.), Kroes et al. (2004) made a number of other recommendations for exclusion of particular groups from the TTC approach. They recommended exclusion of polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls, along with heavy metals, because they are known to accumulate in the body. Other non-essential metals in elemental, ionic or organic forms were also recommended to be excluded because they were not included in the original database of Munro et al. (1996), nor are inorganic substances covered by the structural scheme of Cramer et al. (1978). Proteins were also recommended to be excluded since they were not included in the Munro et al. (1996) database, and their potential for allergenicity and the potent biological activities of some peptides make them unsuitable for the TTC approach.

Evaluation of endpoints of specific concern

The TTC concept and the TOR approach for food contact materials were discussed by the EC Scientific Committee for Food in 1996 and one of the issues raised was whether, for certain endpoints of specific concern, toxic effects might occur at low dose levels which would not be covered by the human exposure thresholds derived by Munro et al. (1996). In particular, concerns were raised about whether effects on the nervous system, immune system, endocrine system and development would be absent at the human exposure threshold values (SCF, 1998). Although the original database published by Munro et al. in 1996 did include some studies measuring these endpoints of specific concern, they were insufficient in number to provide a robust answer to the question of potential low-dose effects.

An Expert Group was therefore set up by ILSI Europe to examine this question in more detail (Kroes et al., 2000). Expanded databases were developed for the toxicological endpoints of neurotoxicity (82 substances), immunotoxicity (37 substances), developmental neurotoxicity (52 substances) and developmental toxicity (81 substances). They were analysed to see if toxic effects involving these endpoints occurred at lower doses than those for structural Class III substances in the original database of Munro et al. (1996). The analysis showed there was no difference between the cumulative NOELs for Class III substances and those for the four selected endpoints, other than for neurotoxicity. The cumulative distribution of NOELs for neurotoxicity was not only lower than those of the other selected endpoints, but it was also clearly lower than that for structural Class III compounds. Consistent with the earlier findings of Cheeseman et al. (1999), the TTC value of 1.5 μg/person per day, based on cancer endpoints, covered all these effects, being 2-3 orders of magnitude lower than the neurotoxicity NOELs divided by a safety factor of 100.

Subsequently Kroes et al. (2004) further explored whether particular neurotoxicants should be considered as a separate class. Using the expanded database from the earlier work (Kroes et al., 2000) and locating the most sensitive indicators of effects that they could find, the NOELs for the most potent neurotoxicants, the organophosphorus compounds (OPs), were plotted separately from the other neurotoxicants. They noted that the 5th percentile NOEL for OPs was lower, by around an order of magnitude, than the corresponding NOEL for other neurotoxicants. The other neurotoxicants resulted in a plot comparable to the Class III chemicals examined by Munro et al. (1996). By applying a safety factor of 100 to the 5th percentile NOEL for OPs, Kroes et al. (2004) derived a human exposure threshold of 18 μg/person per day and recommended that this figure be used for OPs rather than the value of 90 microgrammes/person per day used for other substances in structural Class III.

For references, see list in main text.

APPENDIX B

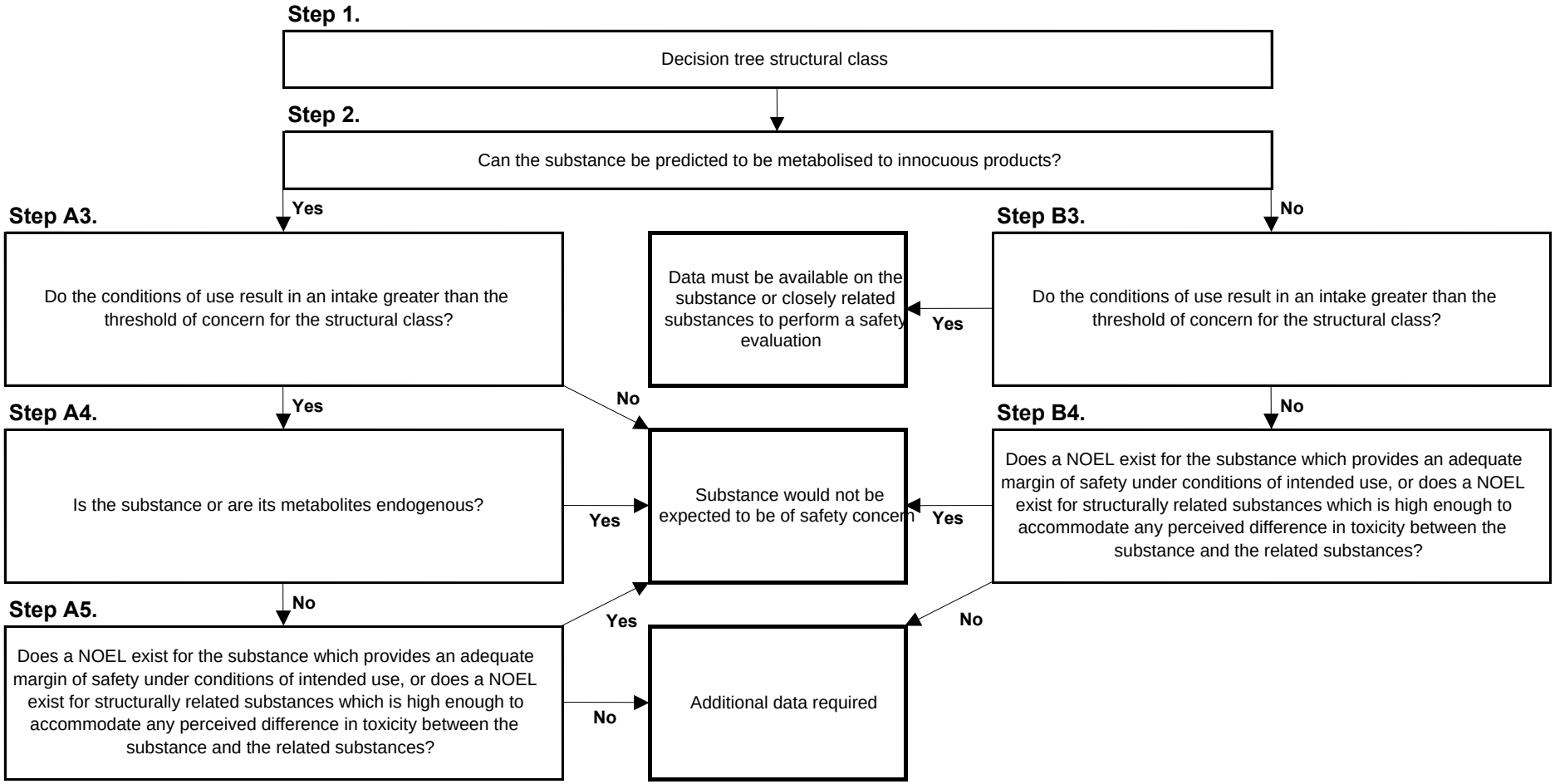
The TTC approach for flavouring substances

The application of the TTC approach for flavouring substances as used by EFSA is illustrated in Figure 1 below. It is a modification of the procedure used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In both procedures, the first consideration is the known or predicted metabolic pathway of the flavouring substance, which asks the question "Can the substance be predicted to be metabolised to innocuous products?". If "Yes" then the substance goes down the "A" side of the procedure. If "No", it goes down the "B" side. For a substance going down the A side, provided the intake is below the relevant TTC value for its structural class, it is considered to be of no safety concern. If the intake exceeds the relevant TTC value, but the substance or its metabolites are endogenous, then again it is concluded there is no safety concern. If the substance or its metabolites are not endogenous, then toxicity data are required, either on the substance itself or on a structurally-related substance, which allow a NOEL to be identified that provides an adequate margin of safety. If none of those conditions are met then additional data are required. For a substance going down the B side and for which the estimate of dietary exposure is below the relevant TTC value, in order to conclude there is no safety concern, either toxicity data on the substance itself or on a structurally-related substance are required, allowing a NOEL to be identified that provides an adequate margin of safety, or (in JECFA evaluations) dietary exposure must be below 1.5 µg/day. If these conditions are not met then additional data are required. Similarly, if dietary exposure is above the relevant TTC value then data are required on the substance itself or a closely related substance to perform a safety evaluation (Munro et al., 1999; Renwick, 2004).

The EFSA procedure is similar to that of JECFA, but EFSA does not use the threshold value of 1.5 µg/day, although it should be noted that JECFA does not use that TTC value either if a flavouring substance is known to be genotoxic. EFSA also uses an additional method for estimating dietary exposure to ensure that high consumers are taken into account. EFSA and JECFA are also now using (or propose to use) an additional dietary exposure estimate that reflects consumption by those regularly consuming a particular brand of a flavoured product (WHO, 2009c; EFSA, 2010e).

For references, see list in main text.

Figure 1: Procedure for Safety Evaluation of Chemically Defined Flavouring Substances as used by EFSA



APPENDIX C

EFSA's analysis of NOELs for substances in Cramer class I and III in the Munro et al Database

Verification of the NOELs for substances in the lowest 10th percentile of the Cramer Class I distribution of NOELs

The Scientific Committee examined the critical studies for the substances in the lowest 10th percentile of the Cramer Class I and Cramer Class III distributions of NOELs presented by Munro et al. (1996) to ascertain whether the numerical value of the NOEL cited by Munro et al. could be verified. The results of this evaluation have been described in the main text of the opinion and are summarised in Tables 1 and 2 below. For Cramer Class I, some additional commentary to that in the main text of the opinion is given below.

For isopropyl alcohol the original paper on the critical study is in Russian, but the study is cited in a JECFA monograph (WHO, 1999b). The JECFA description of the study does not identify the overall NOEL for all the effects reported by the original authors. Munro et al. (1996) used the lowest dose tested (0.018 mg/kg bw per day) as the NOEL, commenting that the original authors reported teratogenicity even at this low dose. However, they went on to note that metabolic considerations would not raise suspicion for toxic effects and that other developmental toxicity studies using much higher doses than the critical study did not find any teratogenic effects. Nevertheless Munro et al. (1996) retained this NOEL in the database, in order to be conservative.

The NOEL for triethylene glycol could not be verified as the original reference is to an abstract and we were not able to locate any subsequent full publications. However, from the abstract it is evident that the NOEL could be as high as 550 mg/kg bw per day, not 0.5 mg/kg bw per day as cited by Munro et al. (1996). This is because the doses in the abstract were expressed in units of ml/kg bw/day with 0.5 ml/kg bw per day being the NOEL stated by the original authors. This has probably been erroneously recorded by Munro et al. (1996) as 0.5 mg/kg bw per day, making the NOEL overly conservative for this substance.

The NOELs of 0.6 and 1.4 mg/kg bw per day for 2,6- and 3,4-dimethylphenol, respectively, were taken by Munro et al. (1996) from the IRIS database and were verified by us from the original paper. It was not possible to judge the quality of this study on the two dimethylphenols from the original paper, but it was notable that the study was not conducted to a standard protocol, and that few methodological details were given (no indication of mode of oral administration, no group sizes, no indication of what was examined except from table of results).

The NOEL from the critical study on oleylamine could not be verified because it has been published in abstract only and we were not able to locate any subsequent full publications. The abstract gives the same NOEL of 3 mg/kg bw per day as listed by Munro et al. (1996).

The original paper for riboflavin could not be obtained, but the study is cited in a JECFA monograph (WHO, 1981b). Munro et al. (1996) identified the NOEL as 4 mg/kg bw per day. The JECFA monograph states that the doses were 4 and 40 ppm given in the diet to young rats and no effects were identified. Using standard conversion factors, 40 ppm in the diet equates to an oral intake of around 4 mg/kg bw per day, which was the NOEL listed by Munro et al. (1996).

The original paper was obtained for isoamyl acetate. The conduct of the study was comparable to that of a 90-day repeated-dose OECD protocol and the NOEL of 4.7 mg/kg bw per day used by Munro et al. (1996) was verified.

The NOEL for ascorbic acid could not be verified from the original study report on developmental toxicity as it is unpublished, neither could it be verified from the brief description of the study by JECFA (WHO, 1981a). However, it is evident that Munro et al. (1996) used the lowest dose tested of 5.5 mg/kg bw per day as the NOEL. This was a conservative approach as no significant effects were

reported in either the critical study or another developmental toxicity study, in both of which the highest doses tested were >500 mg/kg bw per day.

The original publication on the critical study for ethyl acrylate showed that it was well-designed and well-reported. The NOEL of 8.4 mg/kg bw per day used by Munro et al. (1996) was obtained by utilising standard conversion factors for rat body weight and food consumption to derive the average amount of test substance consumed. Using the actual data on body weight and food consumption from the original publication, a NOEL of 5.6 mg/kg bw per day can be derived, which is slightly more conservative than the NOEL used by Munro et al. (1996).

The original publication on the critical study for methyl methacrylate showed that it was well-designed and well-reported. The NOEL of 8.4 mg/kg bw per day used by Munro et al. (1996) was obtained by utilising standard conversion factors for rat body weight and food consumption to derive the average amount of test substance consumed. The designated NOEL by Munro was based on reduced food consumption in rats but only fluid consumption was reduced, and the effects seen on body weight were reversible. As no treatment related effects were found, the NOEL from this study was found to be 2000 ppm, the highest dose tested. Using the actual data on body weight and food consumption from the original publication, a NOEL of 146.5 mg/kg bw per day can be derived, which is less conservative than the NOEL used by Munro et al. (1996).

For dodecyl gallate the original paper is in Russian, but the study is cited in a JECFA monograph (WHO, 1993). The JECFA description of the study indicates the same NOEL of 10 mg/kg bw/day as listed by Munro et al. (1996). The NOEL is verified.

The original report on the critical study on ionone could not be obtained as it is unpublished, but the study is cited in a JECFA monograph (WHO, 1984a). The JECFA description of the study indicates it was well-conducted as it was specifically designed to investigate possible haematological and renal effects indicated in a previous subchronic study. The JECFA description of the study indicates the same NOEL of 10 mg/kg bw per day as listed by Munro et al. (1996). The NOEL is verified based on the JECFA analysis.

The original paper on the critical study for 4-methyl-1-phenylpentan-2-ol showed that it was well-designed and well-reported. The NOEL of 10 mg/kg bw/day reported by the study authors and used by Munro et al. (1996) was verified.

The original paper on the critical study for 2-phenyl-1-propanol showed that it was well-designed and well-reported 90-day study. The NOEL of 10 mg/kg bw per day reported by the study authors and used by Munro et al. (1996) could not be verified since there were statistically significant reductions in body weight in all treated females, including the lowest dose group of 10 mg/kg bw per day, at all of the 2-weekly time points measured.

The original paper on the critical study for retinol was a non-standard developmental toxicity study in which retinol was given to mice at 0, 10 or 100 mg/kg bw as a single gavage dose on day 11 of gestation. The NOEL of 10 mg/kg bw reported by the authors and used by Munro et al. (1996) was verified. However, in the light of current knowledge on the teratogenicity of retinol, which indicates that duration of exposure can also be important, the design used for the critical study would not be expected to give the lowest NOEL for developmental toxicity and, indeed, other studies in rabbits and humans have indicated that the NOEL for developmental toxicity is lower than 10 mg/kg bw/day, at 2.5 mg/kg bw/day in rabbits, and possibly as low as around 0.05 - 0.1 mg/kg bw/day for humans (SCF, 2002; UK FSA, 2003). Thus the Munro et al. (1996) database is not conservative with respect to the NOEL for retinol.

The original report on the critical 2-year rat study on styrene could not be obtained as it is unpublished, but the study is cited in a JECFA monograph (WHO, 1984b). The JECFA description of

the study indicates it was well-conducted and that the NOEL was 12 mg/kg bw per day, which is the same as that used by Munro et al. (1996).

Table 1. Lowest 10th percentile of substances from the Munro et al. (1996) database in Cramer Class I.

Substance	Code (Munro et al., 1996)	CAS number	NOEL cited by Munro et al. (mg/kg bw/d)	Reference & remarks on citation	Appropriate NOEL for study?*	Endpoint from which the Munro et al. NOEL was derived
Ascorbic acid	7	50-81-7(a)	5.5	Food & Drug Research Laboratories Unpublished 1974 Cited in JECFA 23M WHO Food Add Ser 14	Yes. Not verifiable but is conservative based on JECFA evaluation	Musculoskeletal.
2,6-Dimethylphenol	39	576-26-1	0.60	Veldre & Janes Environ Hlth Perspect 30,141-146,1979 Munro took description of study from IRIS DB #0230	Yes	Multiple effects (body weight, blood pressure and pathology of internal organs)
3,4-Dimethylphenol	40	95-65-8	1.40	Veldre & Janes Environ Hlth Perspect 30,141-146,1979 Munro took description of study from IRIS DB #0230	Yes	Multiple effects (body weight, blood pressure, peripheral blood parameters and pathology of internal organs)
Dodecyl gallate	44	1166-52-5	10	Mikhailova et al Vopr Pitan 2,49,1985 Cited in JECFA 41M WHO Food Add Ser 32	Yes	Multiple effects. Deaths, changes in serum lipids and enzymes, reduction in weight of the spleen and, pathological changes in the liver, kidney, and spleen.
Ethyl acrylate	47	140-88-5	8.40	Borzelleca et al Toxicol Appl Pharmacol 6, 29-36,1964	No. Based on measured body weights and food consumption data the NOEL should be lower (5.6 mg/kg bw per day).	Food consumption.

Ionone	80	8013-90-9	10	Ford et al Unpublished (RIFM) 1983 Cited in JECFA 28 WHO Food Add Ser 19	Yes	Multiple effects reduced weight gain, reduced food consumption reduced serum glucose concentrations increased water intakes and mild renal functional changes. No histological changes were evident in the kidneys or livers.
Isoamyl salicylate	82	87-20-7	4.7	Drake et al Food Cosmet Toxicol 13, 185-193,1975 Munro took from RIFM DB	Yes	Organ weight changes. Increased relative kidney weights and adverse effects on kidney function
Isopropyl alcohol	85	67-63-0	0.018	Antonova & Salmina Gig Sanit 1, 8-11, 1978 Cited in SCF ADI 11th Series Report 1981 Cited in JECFA 51M WHO Food Add Ser 42 only for flavourings use	Yes. It was the NOEL from the study, but EFSA is aware that later studies on developmental toxicity using much higher doses did not find evidence of teratogenicity	Teratogenic
Methyl methacrylate	95	79-41-4	8.40	Borzelleca et al Toxicol Appl Pharmacol 6, 29-36, 1964	No. Food consumption was not reduced so NOEL should be higher (146.5 mg/kg bw per day)	Food consumption.
Methyl-1-phenylpentan-2-ol, 4-	97	38502-29-3	10	Ford et al Food Chem Toxicol 21, 441-447, 1983 Munro took from RIFM DB	Yes	Blood effects. a decrease in serum glucose in males. The authors considered this of questionable toxicological significance, however effect was also seen in highest dose group.
Oleylamine	105	1838-19-3	3.0	Mercieca et al Teratology 41, 577, 1990 Munro took abstract from DART DB	Yes, based on abstract	Multiple effects. Maternal toxicity (body weight loss, reduced food consumption), no developmental toxicity was observed.
Phenyl-1-propanol, 2-	109	698-87-3	10	Gaunt et al Food Chem Toxicol 20, 519-525, 1982	No. Females of all dose groups including 10 mg/kg bw per day had statistically significant reduced body weights from week 4 onwards	Liver and kidney weights

					so no NOEL can be identified	
Retinol	115	68-26-2	10	Eckhoff et al Toxicol Lett 48, 171, 1989 From DART DB	Yes. This was the NOEL from the study in the mouse, but data from the rabbit gives a NOEL around an order of magnitude lower for teratogenic effects (Rosa et al., 1986).	Teratogenic
Riboflavin	116	83-88-5	4.0	Le Clerc Ann Nut Aliment 23, 111-120, 1974 Cited in JECFA 25M WHO Food Add Ser 16	Yes, based on JECFA evaluation	No effects, NOEL highest dose tested
Styrene	124	100-42-5	12	Chemical Manufacturers' Association, Litton Bionetics 1980 Cited in JECFA 28 WHO Food Add Ser19	Yes, based on JECFA evaluation	Body weight
Triethylene glycol	132	112-27-6	0.50	Neeper-Bradley et al Toxicologist 14, 160, 1994 Society of Toxicology abstract, Munro took abstract from DART DB	No, based on abstract, NOEL likely much higher because units were in mL/kg bw/day, not mg/kg bw per day.	Teratogenic.

*The column headed "Appropriate NOEL for study?" indicates whether the NOEL was confirmed in our analysis.

Verification of the NOELs for substances in the lowest 10th percentile of the Cramer Class III distribution of NOELs

The commentary on Class III can be found in the main text (chapter 4.2.3.2).

Table 2: Lowest 10th percentile of substances from the Munro et al. (1996) database in Cramer Class III.

Substance	Code (Munro et al., 1996)	CAS number	NOEL cited by Munro et al. (mg/kgbw/d)	Reference & remarks on citation	Appropriate NOEL for study?*	Endpoint from which the Munro et al. NOEL was derived
Acrylamide	30	79-06- 1	0.2	Burek et al., 1980	Yes	Neurotoxicity
Aldicarb	35	16-06-03	0.3	Union Carbide, 1968	Yes to limited extent (from IRIS)	Reproductive toxicity
Avermectin B_1	62	65195-55-3	0.03	Merck & Co., 1985	Yes to limited extent (from IRIS)	Teratogenicity
Azinphos methyl	64	86-50-0	0.18	Huntingdon Research Centre, 1966	No. Insufficient detail in JMPR report (1969)	Haematological effects (details not available)
Bidrin (Dicrotophos)	77	141-66-2	0.1	Shell Chemical Co., 1965	Yes to limited extent (from IRIS)	Reproductive toxicity (decreased pup survival)
Chlordane	106	57-74-9	0.055	Velsicol Chemical, 1983	Yes to limited extent (from IRIS)	Hepatotoxicity
Coumaphos	130	56-72-4	0.4	Doull et al., 1960	No. Insufficient detail in JMPR report (1969)	Multiple effects (no further information could be retrieved)
Cyhalothrin	137	68085-85-8	0.5	Imperial Chemicals Industries, 1984	Yes to limited extent (from IRIS)	Body weight reduction
Cypermethrin	138	523 15-07-8	0.5	ICI Americas, Inc., 1979	Yes to limited extent (from IRIS)	Body weight reduction
2,4-Dichlorophenol	162	120-83-2	0.3	Exon and Keller, 1985	Yes to limited extent (from IRIS)	Multiple effects (Only decreased delayed hypersensitivity cited in IRIS)
Dichlorvos	166	62-73-7	0.23	Shell Chemical Co., 1967	Yes to limited extent (from IRIS)	Multiple effects (Cholinesterase [type not stated, but not brain] inhibition and hepatocellular vacuolation)
Dieldrin	168	60-57-1	0.005	Walker er al., 1969	Yes	Hepatotoxicity

22,23-Dihydroavermectin-B_{1a}, (Ivermectin)	173	71827-03-7	0.2	Merck & Co., 1979	Yes to limited extent (from JECFA monograph)	Neurotoxicity
22,23-Dihydroavermectin-B_{1b}, (Ivermectin)	174	71827-03-7	0.4	Merck & Co., 1979	Yes to limited extent (from JECFA monograph)	Non-specific effects
Dimethoate	178	60-51-5	0.05	American Cyanamid, 1986a	Yes to limited extent (from IRIS)	Neurotoxicity
m-Dinitrobenzene	185	99-65-0	0.4	Cody et al., 1981	Yes	Organ weight changes (increased spleen weights)
Diquat	194	85-00-7	0.19	Chevron, 1985b	Yes to limited extent (from IRIS)	Ocular effects (minimal lens opacity and cataracts)
Disulfoton	195	98-04-4	0.05	Mobay Chemical, 1975	Yes to limited extent (from IRIS)	Multiple effects (inhibition of RBC ChE and brain ChE; males: increased mortality; increase in absolute and relative weights of spleen, liver, and pituitary, decrease in absolute and relative weights of brain and seminal vesicles; females: decrease in absolute and relative weight of kidneys
Ethion	206	563-12-2	0.2	FMC Corp., 1985	Yes to limited extent (from IRIS)	Haematological effects (plasma ChE inhibition in females)
Ethyl *p*-nitrophenyl phenylphosphorothioate	208	2104-64-5	0.25	Moribani, Nissan, du Pont, Velsicol, 1986	Yes to limited extent (from IRIS)	Haematological effects (decreased plasma ChE activity and decreased RBC, hemoglobin, and hematocrit in both sexes. Also decreased brain ChE activity, decreased female growth)
Fenamiphos	215	22224-92-6	0.1	Mobay Chemical, 1982	Yes to limited extent (from IRIS)	Body weight reduction

Fonofos	226	944-22-9	0.5	Stauffer Chemical Co., 1968	Yes to limited extent (from IRIS)	Haematological effects (plasma and RBC ChE inhibition)
Glufosinate-ammonium	228	77182-82-2	0.4	Hoescht, 1982	Yes to limited extent (from IRIS)	Organ weight changes (increase in absolute and relative kidney weights was noted in males)
Haloxyfop-methyl	230	69806-40-2	0.005	Dow Chemical, 1985	Yes to limited extent (from IRIS)	Organ weight changes (decreased relative kidney weights)
Heptachlor	232	76-44-8	LEL 0,25 rat 2y 5 ppm liver/bw weight NOEL 0.15 3 ppm	Velsicol Chemical, 1955. Available from EPA, IRIS. Accession number 0243	Yes to limited extent (from IRIS) In IRIS also present rat 2y: LOEL 0,25 liver/bw weight and NOEL 0.15 3 ppm JMPR and INCHEM set lower ADIs	Reproductive toxicity
Heptachlor epoxide	233	1024-57-3	0,25 rat 3 gen repr 5 ppm	Velsicol Chemical, 1959. Available from EPA, IRIS. Accession number 0160	Yes to limited extent (from IRIS)	Reproductive toxicity
Hexachloro benzene	235	118-74-1	0,08 rat 130 w F0 + F1 1.6 ppm (0.08)	Arnold et al., 1985. Food Chem Toxicol 23, 779-793. Available From EPA, IRIS. Accession number 0374	Yes to limited extent (from IRIS) and Abstract of the original paper	Hepatotoxicity
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	241	121-82-4	0,3 rat 2y	U.S DOD, 1983. Available from Defense Tech Center. From EPA, IRIS. Accession number 0313	Yes to limited extent (from IRIS)	Multiple organs effects

Merphos (akatributyl phosphorotri thioite)	272	150-50-5	0,1 rat 112 d neurotox Munro UF 300	Virginia Carolina Chemical Corp., 1985. Available from EPA. IRIS. Accession number 0366	Yes to limited extent (from IRIS)	Haematological effects (RBC ChE inhibition)
Merphos oxide	273	78-48-8	0.25 rat 2y	Mobay Chemical, 1969. Available from EPA, IRIS. Accession number 0367	Yes to limited extent (from IRIS)	Neurotoxicity (Brain ChE inhibition)
Methidathio n	277	950-37-8	0,2 rat 2y 4 ppm	Ciba, 1986. Available from EPA, IRIS. Accession number 0341	Yes to limited extent (from IRIS)	Multiple effects (RBC, brain ChE inhibition and alopecia)
Methyl parathion	283	298-00-0	0,025 rat 2y 0.5 ppm	Monsanto Co., 1984. Available from EPA, IRIS. Accession number 0174	Yes to limited extent (from IRIS)	Organ weight changes
Mirex	292	2385-85-5	0,17 mice 18 m 1 ppm	Fulfs et al., 1977. Ecotoxicol Environ Saf 1: 327. Available from from EPA, IRIS. Accession number 0251	Yes to limited extent (from IRIS)	Hepatotoxicity
Molinate	293	2212-67-1	0,2 rat fertility (time?)	Stauffer Chemical Co., 1981. Available from EPA, IRIS. Accession number 0298	Yes to limited extent (from IRIS)	Reproductive toxicity
Naled	296	300-76-5	0,2 rat 2y	Chevron, 1984a. Available from EPA, IRIS. Accession number 0175	Yes to limited extent (from IRIS)	Non-specific effects (brain ChE inhibition)

Ozadiazon	317	19666-30-9	0,5 10 ppm	Rhone-Poulenc, 1981c. Available from EPA, IRIS. Accession number 0253	Yes to limited extent (from IRIS)	Multiple effects (serum proteins and liver weights)
Oxyfluorfen	320	42874-03-3	0,3 mouse 20 m 2 ppm	Rohm and Haas Co., 1977. Available from EPA, IRIS. Accession number 0084	Yes to limited extent (from IRIS)	Hepatotoxicity
Patulin	327	149-297-1	0,04 calculated	Becci et al., 1981. J Appl Toxicol 1: 256-261. Cited in: Additives and Contaminants 35th Meeting of JECFA. WHO Food Additives Series, No. 26	Yes from the JECFA monograph	Body weight reduction
Photodieldrin	344	13366-73-9	0,35 rat 59-80 w 7.5 ppm	NCI, 1977. National Cancer Institute Technical Report No. 17	Yes to limited extent (from IRIS)	Neurotoxicity
Pirimphos-methyl	349	29232-93-7	0,5 dog 2 y ChE LEL	ICI Americas, Inc., 1973. Available from EPA, IRIS. Accession number 0257	Yes to limited extent (from IRIS)	Haematological effects (plasma ChE depression)
Quinalphos	372	13593-03-8	0,03 mouse 18 m	Sandoz, Inc., 1983 1980. Available from EPA, IRIS. Accession number 0082	Yes to limited extent (from IRIS)	Haematological effects (plasma ChE depression)
Rotenone	379	83-79-4	0,38 rat 2 gen 7.5 ppm	U.S. Fish and Wildlife Service, 1983. Available from EPA, IRIS. Accession number 0344	Yes to limited extent (from IRIS)	Reproductive toxicity (Reduced pup weight)

Sodium fluoroacetate	385	62-74-8	0,05 rat 13 w UF 3000 Munro UF 300	U.S. EPA, 1988b. Available from EPA, IRIS. Accession number 0469	Yes to limited extent (from IRIS)	Multiple effects (Increased heart weight in females and males; decreased testis weight and altered spermatogenesis in males)
Terbutryn	399	886-50-0	0,1 rat 2 y 2 ppm	Ciba, 1980b. Available from EPA, IRIS. Accession number 0285	Yes to limited extent (from IRIS)	Haematological effects (hemoglobin and erythrocytes decrease)
Tetrachloro benzene, 1,2,4,5-	401	95-94-3	0,34 rat 13 w UF 1000 Munro UF 300	Chu et al., 1984. Drug Chem Toxicol 7: 113. Available from EPA, IRIS. Accession number 0107	Yes to limited extent (from IRIS)	Kidney toxicity
Tetraethyldi thiopyropho sphate	409	3689-24-5	0,5 rat 3 m 10 ppm (0.5) UF 1000 Munro UF 300	Kimmerle et al., 1974. Arch Toxicol 33: 1-16 Available from EPA, IRIS. Accession number 0330	Yes to limited extent (from IRIS)	Haematological effects (plasma ChE depression)
Trenbolone acetate	422	10161-34-8	0,044 (0.025) rat F0 + F1 0.5 ppm	James P, Smith JA, Parker CA 1986 Unpublished report Huntingdon	Yes from the JECFA monograph	Reproductive toxicity
Trenbolone hydroxide, 17a-	423		0,04 Munro UF 300		Yes from the JECFA monograph	Haematological effects
Tridiphane	436	58138-08-2	0,33 rat 2 gen rep 5 ppm	Dow Chemical, 1984. Available from EPA, IRIS. Accession number 0124	Yes to limited extent (from IRIS)	Reproductive toxicity

Zeranol	448	55331-29-8	0,02 (0.0125) rat 2y 0.25 ppm NHEL Monkey ovariectom-ised (0.05)	Everett et al., 1987. Unpublished report. Cited in: JECFA, 1988.Toxicol ogical Evaluation of Certain Veterinary Drug Residues in Food. 32nd Meeting of the JECFA. WHO Food Additives Series, No. 23	Yes from the JECFA monograph	Ovarian toxicity

*The column headed "Appropriate NOEL for study?" indicates whether the NOEL was confirmed in our analysis.

For references, see list in main text and reference list in Munro et al. 1996.

APPENDIX D

Establishing a TTC value for substances with anti-cholinesterase activity

In total, 93 ADIs for 59 OPs and 27 ADIs for 14 carbamates are listed in the EFSA database on pesticides and are shown in Table 1 below.

Table 1: Neurotoxicity data used to establish ADIs and ARFDs on organophosphorus and carbamates from the EFSA database on pesticides

Pesticide class	Compound_name	ADI mg/kg bw/d	Safety factor	Source	Year	Study	Usage category*
organophosphate	Acephate	0.03	10	JMPR	2005	28 d human	IN
carbamate	Aldicarb	0.003	10	JMPR	1995	acute human	IN+NE+AC
organophosphate	Azinphos-methyl	0.005	100	DE	2008	multigeneration rat	IN+AC
organophosphate	Azinphos-methyl	0.005	100	JMPR	1991	rat multigeneration	IN+AC
organophosphate	Azinphos-methyl	0.03	10	JMPR	2007	30 d human	IN+AC
organophosphate	Azinphos-methyl	0.005	100	SCFCAH March 2006 (Draft review report)	2006	rat multigeneration	IN+AC
carbamate	Bendiocarb	0.004	100	JMPR	1984	2 yr rat	IN
carbamate	Benfuracarb	0.01	100	EFSA	2006	90 d dogs, rat multigeneration	IN+NE
carbamate	Benfuracarb	0.01	100	EFSA	2009	overall NOAEL dogs, 2 generation rats	IN+NE
organophosphate	Bromophos	0.04	10	JMPR	1977	28 d human	IN
organophosphate	Bromophos-ethyl	0.003	100	JMPR	1975	2 yr dog	IN
organophosphate	Cadusafos (aka ebufos)	0.0004	100	EFSA	2006	2 yr rat	IN+NE
organophosphate	Cadusafos (aka ebufos)	0.0004	100	EFSA	2008	2 yr rat	IN+NE
organophosphate	**Cadusafos (aka ebufos)**	**0.0003**	**100**	**JMPR**	**1991**	**rat multigeneration**	**IN+NE**
carbamate	Carbaryl	0.0075	2000	EFSA	2006	2 yr mouse	IN+PG
carbamate	Carbaryl	0.008	2000	JMPR	2001	2 yr rat	IN+PG
carbamate	Carbofuran	0.001	100	EFSA	2006	1 yr dog	IN+NE+AC
carbamate	Carbofuran	0.001	25	JMPR	2008	rat, acute toxicity	IN+NE+AC
carbamate	**Carbofuran**	**0.00015**	**200**	**PRAPeR phone conference January 2009**	**2009**	**acute neurotoxicity**	**IN+NE+AC**
organophosphate	Carbophenothion	0.0005	50	JMPR	1980	2 yr rat	IN+AC
carbamate	Carbosulfan	0.005	100	DAR	2009	rat acute neurotoxicity	IN+NE
carbamate	Carbosulfan	0.01	100	EFSA	2006	2 yr rat	IN+NE
carbamate	Carbosulfan	0.005	100	EFSA	2009	Rat, acute neurotoxicity	IN+NE
carbamate	Carbosulfan	0.01	100	JMPR	2003	2 yr rat	IN+NE
organophosphate	Chlorfenvinphos	0.0005	100	JMPR	1994	rat multigeneration	IN

organophosphate	Chlorpyrifos	0.01	100	COM	2005	2 yr rat, 2 yr mouse, 2 yr dog	IN+AC
organophosphate	Chlorpyrifos	0.01	100	JMPR	1982	9 d human	IN+AC
organophosphate	Chlorpyrifos-methyl	0.01	100	COM	2005	2 yr rat	IN+AC
organophosphate	Chlorpyrifos-methyl	0.01	10	JMPR	1992	28 d human	IN+AC
organophosphate	**Demeton-S-methyl**	**0.0003**	**100**	**JMPR**	**1989**	**2 yr rat**	**IN+AC**
organophosphate	**Demeton-S-methyl sulphone**	**0.0003**	**100**	**JMPR**	**1989**	**3 yr rat**	**IN**
organophosphate	**Diazinon**	**0.0002**	**100**	**EFSA**	**2006**	**90 d dog, 1 yr dog**	**IN+AC**
organophosphate	Diazinon	0.005	100	JMPR	2006	90 d rat	IN+AC
organophosphate	Dichlorvos	0.004	10	JMPR	1993	human	IN+AC
organophosphate	Dimethoate	0.001	100	EFSA	2006	2 yr rat, rat multigeneration, rat neurotoxicity, rat developmental neurotoxicity	IN+AC
organophosphate	Dimethoate	0.002	500	JMPR	1996	rat multigeneration	IN+AC
organophosphate	Dioxathion	0.0015	100	JMPR	1968	90 d rat neurotoxicity	IN
organophosphate	**Disulfoton**	**0.0003**	**100**	**JMPR**	**1996**	**2 yr dog**	**IN**
carbamate	Ethiofencarb	0.1	100	BE	1982	28 d rat	IN
carbamate	Ethiofencarb	0.1	100	JMPR	1982	28 d rat	IN
organophosphate	Ethion (aka diethion)	0.002	100	JMPR	1990	rat developmental	IN+AC
organophosphate	Ethoprophos	0.0004	100	EFSA	2006	2 yr rat	IN+NE
organophosphate	Ethoprophos	0.0004	100	JMPR	1999	2 yr rat, rat multigeneration	IN+NE
organophosphate	Etrimfos	0.003	100	JMPR	1986	2 yr rat	IN+AC
organophosphate	Fenamiphos (aka phenamiphos)	0.0008	100	EFSA	2006	1 yr dog	NE
organophosphate	Fenamiphos (aka phenamiphos)	0.0008	100	JMPR	1997	1 yr dog	NE
organophosphate	Fenitrothion	0.005	100	EFSA	2006	2 yr rat	IN+AC
organophosphate	Fenitrothion	0.005	100	JMPR	2000	2 yr dog	IN+AC
organophosphate	Fenitrothion	0.006	100	JMPR	2007	90 d rat, 6 mo rat, 2 yr rat (overall NOAEL)	IN+AC
organophosphate	Fenthion	0.007	10	DE	2001	human	IN
organophosphate	Fenthion	0.007	10	ECCO	2001	28 d human	IN
organophosphate	Fenthion	0.007	10	JMPR	1995	25 d human	IN
organophosphate	Fonofos	0.002	100	BE	1986	2 yr dog	IN
carbamate	Formetanate	0.004	100	EFSA	2006	1 yr dog	IN+AC
organophosphate	Fosthiazate	0.004	100	COM	2003	2 yr rat	NE
organophosphate	Heptenophos	0.003	100	BE	1987	2 yr dog	IN
organophosphate	Heptenophos	0.002		DE	1997	90 d dog	IN
organophosphate	Isofenphos	0.001	50	JMPR	1986		IN
organophosphate	Isoxathion	0.0125	100	BE	1987	2 yr rat	IN
organophosphate	Malathion	0.03	1000	EFSA	2006	2 yr rat	IN
organophosphate	Malathion	0.03	1000	EFSA	2009	2 yr rat	IN
organophosphate	Malathion	0.3	100	JMPR	1997	2 yr rat	IN
organophosphate	Mecarbam	0.002		JMPR	1986	metabolism, delayed neurotoxicity	IN+AC

organophosphate	Mecarbam	0.0005	200	Scientific Committee	1995	rat multigeneration	IN+AC
organophosphate	Methacrifos	0.006	10	JMPR	1990	human	IN
organophosphate	Methamidophos	0.001	100	COM	2007	2 yr rat	IN+AC
organophosphate	Methamidophos	0.004	25	JMPR	2002	2 yr rat	IN+AC
organophosphate	Methidathion	0.001	100	JMPR	1992	90 d dog, 1 yr dog, 2 yr dog	IN+AC
carbamate	Methiocarb (aka mercaptodimethur)	0.013	100	EFSA	2006	90 d dog	IN+MO+RE
carbamate	Methiocarb (aka mercaptodimethur)	0.02	100	JMPR	1998	2 yr dog	IN+MO+RE
carbamate	Methomyl	0.0025	100	EFSA	2006	rat acute neurotoxicity	IN
carbamate	Methomyl	0.0025	100	EFSA	2008	rat acute neurotoxicity	IN
carbamate	Methomyl	0.02	5	JMPR	2001	human	IN
organophosphate	**Mevinphos**	**0.00025**	**100**	**BE**	**2001**	**90 d rat, 2 yr rat**	**IN+AC**
organophosphate	Mevinphos	0.0008	200	JMPR	1996	30 d human	IN+AC
organophosphate	Monocrotophos	0.0006	10	JMPR	1993	30 d human	IN+AC
organophosphate	Naled	0.002	100	DAR	2004	2 yr rat, 1 yr dog	IN+AC
organophosphate	**Omethoate**	**0.0003**	**100**	**EFSA**	**2006**	**rat multigeneration, 2 yr rat**	**IN+AC**
carbamate	Oxamyl	0.001	100	EFSA	2005	rat acute neurotoxicity	IN+NE
carbamate	Oxamyl	0.009	10	JMPR	2002	acute human	IN+NE
organophosphate	**Oxydemeton-methyl**	**0.0003**	**100**	**EFSA**	**2006**	**2 yr rat**	**IN+AC**
organophosphate	**Oxydemeton-methyl**	**0.0003**	**100**	**JMPR**	**1989**	**3 yr rat**	**IN+AC**
organophosphate	Parathion	0.0006	100	DE	2002	90 d rat neurotoxicity	IN+AC
organophosphate	Parathion	0.0006	100	ECCO 100	2001	90 d rat neurotoxicity	IN+AC
organophosphate	Parathion	0.004	100	JMPR	1995	2 yr rat	IN+AC
organophosphate	Parathion-methyl	0.001	100	DE	2002	2 yr rat	IN+RE
organophosphate	Parathion-methyl	0.001	100	ECCO 127	2002	2 yr rat	IN+RE
organophosphate	Parathion-methyl	0.003	100	JMPR	1995	2 yr rat	IN+RE
organophosphate	Phenthoate	0.003		JMPR	1984		IN
organophosphate	Phorate	0.0007	100	JMPR	2004	2 yr rat, 13 wk rat, 1 yr dog	IN
organophosphate	Phosalone	0.01	100	EFSA	2006	1 yr dog	IN+AC
organophosphate	Phosalone	0.02	100	JMPR	1997	2 yr rat	IN+AC
organophosphate	Phosmet	0.003	300	EFSA	2006	2 yr mouse	IN
organophosphate	Phosmet	0.01	100	JMPR	1994	rat multigeneration	IN
organophosphate	Phosphamidon	0.0005	100	DE	1991	2 yr rat	IN+AC
organophosphate	Phosphamidon	0.0005	100	JMPR	1986	2 yr rat	IN+AC
organophosphate	Phoxim	0.001		IT			IN
organophosphate	Phoxim	0.004	100	JECFA	1999	2 yr dog	IN
carbamate	Pirimicarb	0.035	100	EFSA	2006	1 yr dog	IN
carbamate	Pirimicarb	0.02	100	JMPR	2004	90 d dog, 2 yr dog	IN
organophosphate	Pirimiphos-methyl	0.004	100	EFSA	2005	2 yr rat, 2 yr dog, human data	IN
organophosphate	Pirimiphos-methyl	0.03	10	JMPR	1992	28 d human, 58 d human	IN

organophosphate	Profenofos	0.01	100	JMPR	1990	rat multigeneration	IN
organophosphate	Profenofos	0.03	100	JMPR	2007	90 d dog, 6 mo dog, 1 yr dog (overall NOAEL)	IN
carbamate	Promecarb	0.05		BE			IN
organophosphate	Propanil	0.02	100	BE		2 yr rat	HB
organophosphate	Propanil	0.03	300	DAR	2006	2 yr rat	HB
organophosphate	Propanil	0.03	300	DAR	2010	2 yr rat	HB
organophosphate	Propanil	0.005		IT			HB
organophosphate	Propanil	0.03	300	IT	2006	2 yr rat	HB
carbamate	Propoxur	0.02		JMPR	1989		IN
organophosphate	**Prothiofos**	**0.0001**	**100**	**DE**	**1998**	**1 yr dog**	**IN**
organophosphate	Pyrazophos	0.001	100	ECCO 73	1999	2 yr dog	FU
organophosphate	Pyrazophos	0.004	100	JMPR	1992	2 yr dog, rat multigeneration	FU
organophosphate	Sulfotep	0.001	10	DE	1990	90 d dog	IN+AC
organophosphate	Terbufos	0.0006	100	JMPR	2003	1 yr rat, 90 d rat neurotoxicity, rat multigeneration, 1 yr dog	IN
organophosphate	Tetrachlorvinphos	0.05	100	BE	1988	2 yr dog	IN
organophosphate	Thiometon	0.003	50	JMPR	1979	2 yr dog, rat multigeneration	IN+AC
organophosphate	Thiometon	0.001		NL			IN+AC
organophosphate	Tolclofos-methyl	0.064	100	EFSA	2005	2 yr mouse	FU
organophosphate	Tolclofos-methyl	0.07	100	JMPR	1994	2 yr mouse	FU
organophosphate	Triazophos	0.001	10	JMPR	2002	3 wk human	IN+AC
organophosphate	Trichlorfon	0.045	100	AT	2006	2 yr rat	IN
organophosphate	Trichlorfon	0.045	100	DAR		2 yr rat	IN
organophosphate	Trichlorfon	0.002	100	JMPR	2003	human	IN
organophosphate	Trichlorfon	0.002		NL			IN
organophosphate	Vamidothion	0.008	10	JMPR	1988	3 wk human	IN+AC

*Usage category: IN = insecticide, AC = acaricide, FU = fungicide, RO = rodenticide, MO = molluscicide, NE = nematocide, RE = repellent, HB = herbicide. In **bold**, values at or below the proposed threshold for neurotoxicity.

From Table 1 above, substances with ADIs at or below the proposed TTC value for OPs of 18 µg/person per day (equivalent to 0.3 µg/kg bw per day) were extracted and are listed in Table 2 below. For some of the substances, more than one ADI has been allocated, some of which are above the proposed TTC threshold value; these are listed as well in Table 2. Some of the effects for the substances listed that determine the ADI are related to endpoints other that neurotoxicity, but they are listed for completeness.

Table 2: Organophosphate and carbamate ADIs at or below the proposed TTC threshold for OPs

Name (substance group)	ADI (mg/kg bw)	Study type/ Effects on which ADI is based	LOAEL/ NOAEL ratio	Safety factor	Source	Year
Cadusafos (organo phosphate) *	**0.0003**	Multi-generation rat: NOAEL: 0.5 ppm (0.025 mg/kg bw/d) LOAEL: 5 ppm ↓ reduced bw in F_0 and F_1, m+f	10	100	JMPR	1991
Cadusafos (organo phosphate)	0.0004	2-year rat: NOAEL 1 ppm (0.045 mg/kg bw/d) LOAEL: 5 ppm ↓ plasma and RBC** AChE** *m+f, ↓ locomotion f	5	100	EFSA	2008
Demeton-S-methyl (organo phosphate)	**0.0003**	2 year rat: 2 studies group ADI NOAEL: 1 ppm (0.03 mg/kg bw/d) LOAEL: 5 ppm ↓ brain AChE	5	100	JMPR	1989
Demeton-S-methyl sulphone (organo phosphate)	**0.0003**	2 year rat: 2 studies group ADI NOAEL: 1 ppm (0.03 mg/kg bw/d) LOAEL: 5 ppm ↓ brain AChE	5	100	JMPR	1989
Diazinon (organo phosphate)	**0.0002**	90- day dog: NOAEL: 0.5 ppm (0.02 mg/kg bw/d) LOAEL: 150 ppm: ↓ bw gain m+f, ↓serum AChE m+f, ↓protein levels m, ↓Ca levels f; 1-year dog: NOAEL: 0.5 ppm (0.02 mg/kg bw/d) LOAEL: 150 ppm (4.6 mg/kg bw/d) ↓bw m, ↓food consumption m+f, ↓serum AChE m+f;	300	100	EFSA	2006
Diazinon (organo phosphate)	0.005	90 –day- rat: NOAEL: 0.5 mg/kg bw/d LOAEL: 1 mg/kg bw/d ; ↓AChE in RBC	2	100	JMPR	2006
Disulfoton (organo phosphate)	**0.0003**	2-year dog: NOAEL: 1 ppm (0.03 mg/kg bw/d) LOAEL: 2 ppm ↓serum and RBC AChE	2	100	JMPR	1996
Mevinphos (organo phosphate)	**0.00025**	90-day neurotoxicity rat: NOAEL 0.025 mg/kg bw/d LOAEL: 0.35 mg/kg bw/d ↓ brain, serum and RBC AChE 2-year rat: NOAEL: 0.025 mg/kg bw/d LOAEL: 0.35 mg/kg bw/d ↓ brain AChE	14	100	BE	2001

Name (substance group)	ADI (mg/kg bw)	Study type/ Effects on which ADI is based	LOAEL/ NOAEL ratio	Safety factor	Source	Year
Mevinphos (organo phosphate)	0.0008	30-day human: NOAEL: 1 mg/d or 0.016 mg/kg bw/d LOAEL: 1.5 mg/d ↓ plasma and RBC AChE	1.5	200	JMPR	1996
Omethoate (organo phosphate)	**0.0003**	Multigeneration rat: NOAEL: 3 ppm (0.03 mg/kg bw/d) LOAEL: 18 ppm ↑ post natal loss, ↓ pup weight; ↓ fertility and mating in F_0 and F_1 (effects more pronounced in F_1) 2-year rat: NOAEL 0.03 mg/kg bw/d LOAEL:0.04 mg/kg bw/d ↓ RBC in m (borderline effect - very conservative value)	6 (1.3)	100	EFSA	2006
Oxydemeton-methyl (organo phosphate)	**0.0003**	2-year rat: NOAEL: 0.03 mg/kg bw/d LOAEL: 0.25 mg/kg bw/d: ↓serum AChE m+f	8	100	EFSA	2006
Oxydemeton-methyl (organo phosphate)	**0.0003**	2-year rat: (2 studies - group ADI) NOAEL: 1 ppm (0.03 mg/kg bw/d) LOAEL: 5 ppm ↓ brain AChE	5	100	JMPR	1989
Prothiofos (organo phosphate)	**0.0001**	1-year dog: NOAEL 0.4 ppm (0.01 mg/kg bw/d) LOAEL 300 ppm (7.5 mg/kg bw/d):↓ plasma and RBC AChE	750	100	DE	1989
Carbofuran (carbamate)	**0.00015**	Acute neurotoxicity rat: LOAEL: 0.03 mg/kg bw ↓ brain AChE	-----	200	EFSA	2009
Carbofuran (carbamate)	0.001	1-year dog: NOAEL 0.1 mg/kg bw/d LOAEL: 1 mg/kg bw/d: ↓ RBC AChE, miosis in f	10	100	EFSA	2006
Carbofuran (carbamate)	0.001	Acute toxicity rat: NOAEL: 0.04 mg/kg bw/d LOAEL: 0.3 mg/kg bw/d ↓brain and RBC AChE	7.5	25	JMPR	2008

*Substances with ADIs at or below the proposed TTC value for OPs are listed in bold-face type.
In **bold**, values at or below the proposed threshold for neurotoxicity.
**RBC: red blood cells; ***AChE: acetylcholinesterase. For JMPR references, see http://www.inchem.org/pages/jmpr.html

The toxicological basis on which the ADIs for OPs and carbamates listed in Table 2 were established is described below.

Organophosphates

The ADIs for OPs have been established as follows:

- For cadusafos, an ADI of 0.0003 mg/kg bw has been established by JMPR (1991) based on reduced body weight in dams observed in a rat multi-generation study at a dose exceeding 10 times the NOAEL. In 2008 EFSA established an ADI of 0.0004 mg/kg bw based on inhibition of AChE and reduced locomotion in rats.
- JMPR established a group ADI for demethon-S-methyl and demethon-S-methyl sulphone of 0.0003 mg/kg bw based on inhibition of brain cell AChE at a level exceeding 5 times the NOAEL.
- EFSA established an ADI of 0.0002 mg/kg bw for diazinon based on clinical signs and reduced serum AChE in a 90-day and in a 1-year dog study, the LOAEL exceeding the NOAEL 300 times. In this context it is notable that JMPR (WHO,1999c) recommends considering reduced AChE solely in serum (without parallel inhibition of AChE in brain or red blood cells (RBC) as not adverse.
- JMPR established an ADI of 0.005 mg/kg bw for diazinon based on observations of reduced AChE in RBC.
- For disulfuton an ADI of 0.0003 mg/kg bw has been established by JMPR based on reduced serum and RBC AChE in dogs.
- While for mevinphos the Belgian competent authority established an ADI of 0.00025 mg/kg bw on the basis of inhibition of AChE in brain, serum and RBC in short- and long-term studies in the rat. JMPR established an ADI of 0.0008 mg/kg bw based on similar observations in humans.
- For omethoate an ADI of 0.0003 mg/kg bw has been established based on bases of effects on development and fertility in a multi-generation study and on reduced AChE in RBC of rats.
- For oxydemeton-methyl an ADI of 0.0003 mg/kg bw has been established by EFSA and JMPR, based on inhibition of AChE in serum and brain of rats.
- An ADI of 0.0001 mg/kg bw was established for prothiofos by the German Competent Authority on the basis of reduced AChE in serum and brain in a 1-year dog study, in which a LOAEL/NOAEL ratio of notably 750 could be observed.

Carbamates

The ADIs for carbamates have been established as follows:

- For carbofuran EFSA has established an ADI of 0.00015 mg/kg bw on basis of a LOAEL 0.03 mg/kg bw per day (safety factor of 200) from an acute study in rats in which reduced brain AChE was seen. The ADI previously established by EFSA was 0.001 mg/kg bw based on similar effects seen in dogs. JMPR has established an identical ADI of 0.001 mg/kg bw based on similar observations in an acute rat study.

For references, see list in main text.

APPENDIX E

Exposure assessment in EFSA's Scientific Panels

ANS – Panel on Food Additives and Nutrient Sources Added to Food

A feature specific to additives is that they are intentionally added to food and that their presence in food products is related to the product formulation, which may vary from brand to brand of every single food item. In many cases, formulations are kept confidential and only Maximum Permitted Levels present in the legislation or Typical Use Levels or Upper Use Levels reported by industry are available. The relationship between such levels and actual use levels is very uncertain. For new substances submitted for use as additives, only intended use levels are available and can be used to assess anticipated human dietary exposure.

Few analytical data are currently available in relation to the concentration of additives in foods and beverages ready to be consumed and little is known about the influence of storage and processing on the residues of these substances in food.

The tendency of consumers to repeatedly purchase and consume the same (brands of) food products, termed consumer or brand loyalty, creates a dependency in the form of a positive correlation between the concentrations in different food items consumed by the same consumer. In order to provide a conservative dietary exposure assessment, it may be assumed that consumers are loyal to the brands with the highest concentrations. This introduces a bias, but provides a more accurate estimate for a consumer who is loyal, and also provides higher certainty that the assessment is protective and takes into consideration the consumers who are potentially more exposed to the substance of interest.

Until now the Panel on Food Additives and Nutrient Sources (ANS Panel) in its re-evaluation of food additives (mainly colours) has follows the stepwise approach, which was used in the report of the Scientific Cooperation (SCOOP) Task 4.2. The approach goes from a conservative estimate that forms Tier 1 (screening), to progressively more realistic estimates that form Tier 2 and Tier 3.

At Tier 1, the ANS Panel uses the concept of total food intake in order to determine if proposed maximum use levels of food additives exceed recommended ADI levels; this is referred to as the Budget method. The Budget method is a simple calculation which depicts the worst case exposure scenario based on the physiological upper limits for food and liquid consumption and the assumption that the food additive in question would be present at the maximum permitted levels in a certain proportion of all foods and liquids consumed (Hansen, 1966, 1979; EC, 1998). The Budget method results in an initial crude estimate of exposure and if it shows that the ADI will be exceeded, more precise calculations based on reported use levels and actual food consumption data are performed (Tier 2 and 3).

At Tier 2, refined exposure estimates are performed using maximum permitted use levels.

At Tier 3, refined exposure estimates are performed using maximum reported use levels or analytically determined use levels (if available).

At both Tiers, exposure estimates for children are performed, based on detailed individual food consumption data from 10 European countries. For adults, the Panel uses food consumption data from the UK as being representative of the EU adult consumers.

In the future, exposure assessments for food additives will be based on the EFSA Comprehensive European Food Consumption Database, which gives access to aggregate food categories consumed in 15 European countries (EFSA, 2011b).

Nutrients

For nutrients, which are data rich substances, the application of TTC as a risk prioritisation tool is not considered relevant, therefore the exposure assessment is not discussed here.

CEF – Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids

Food contact materials

Exposure assessments for substances migrating into food from food contact materials (e.g. packaging) differ in a number of ways from other contaminants. The number of substances used for packaging is considerable (e.g. more than 1200 monomers and 1000 additives in plastics manufacturing alone). The level of migration depends on many factors including the duration of contact between food and packaging, and the temperature (during storage, during final preparation, etc.).

Instead of assessing dietary exposure through combination of concentration data in actual foods with consumption data, a model is used to calculate the maximum migration of the substance into food (EFSA, 2008b). In the model, it is assumed a person may consume daily 1 kg of food that is in contact with a particular type of food contact material and that the kg of food is in the form of a cube of surface area 6 dm^2. For fatty foods, a reduction factor up to 5 could be introduced due to the fact that a person is unlikely to consume daily an amount of food containing more than 200 g of pure fat.

The level of migration may be obtained by different methods:

- Most commonly, concentrations in food are estimated from measurements of migration obtained in migration tests with standard food simulants.
- Migration from food contact materials into food is considered to be complete, i.e. 100% of the substance in the food contact material is assumed to migrate into food.
- Theoretical migration modelling with packaging-related rate constants and food-related uptake properties, intended to overestimate migration.

In some rare cases, full dietary exposure assessment is performed based on concentrations measured in foods ready for consumption. However, in these cases and in order to provide a conservative dietary exposure assessment, it may be assumed that consumers are loyal to the brands with the highest concentrations. This introduces a bias, but provides a more accurate estimate for a consumer who is loyal, and also provides higher certainty that the assessment is protective and takes into consideration the consumers who are potentially more exposed to the substance of interest. Therefore, this approach for estimating exposure to food contact material substances requires data which are currently not normally available and is consequently difficult to use.

Flavourings

In the evaluation of flavouring substances, the dietary exposure considered by EFSA within the Procedure to assess their safety has been a per capita estimate, the "Maximised Survey-Derived Daily Intake" (MSDI), based on the annual volume of production reported by the applicant. In addition, the "modified Theoretical Added Maximum Daily Intake" (mTAMDI) was calculated, based on the normal added use levels of the substances as reported by the applicant in the 18 food categories of Annex III of Commission Regulation (EC) No 1565/2000 (European Commission, 2000). Both the MSDI and the mTAMDI approach take into consideration the dietary exposure of a 60 kg adult.

Chronic dietary exposure in adults and children

The Panel has developed a modified approach for estimating high dietary exposures for new flavourings which is in line with the methods that have been used until now for flavourings but addresses some of their limitations. This method called the "Added Portions Exposure Technique" (APET) is used to estimate the dietary exposure for adults and children and is an adaptation of the mTAMDI method. The APET is based on the occurrence levels provided by the applicant in each of the food sub-categories with the exclusion of complementary foods for infants and young children:

1) on the basis of normal occurrence level from added flavourings,

2) on the basis of normal occurrence level from other dietary sources,

3) on the basis of normal combined occurrence levels.

Sub-categories are classified in two groups: "Beverages", and "Solid foods". The APET is calculated by summing the highest potential dietary exposure within each of the two groups and expressed in mg/kg bw per day. For an adult, a body weight of 60 kg is considered and the portions are those established by the JECFA (FAO/WHO, 2008) when developing a similar technique (SPET) (Single Portion Exposure Technique).

Dietary exposure to flavouring substances in infant foods

The diets of infants and young children tend to be less varied than those of older children and adults; an ad hoc method is therefore needed for estimating the exposure in this age group. A specific exposure assessment could be performed based on the model diet of a 12-month young child fed milk and a variety of processed baby foods flavoured with the substance of interest. Due to the high brand loyalty in young children the maximum combined occurrence levels will be considered in this exposure assessment.

The guidance document on the data required for the risk assessment of flavourings to be used in or on foods has recently been published (EFSA, 2010e).

CONTAM – Panel on Contaminants in the food chain

The concentration of both natural (e.g. mycotoxins) and environmental (e.g. heavy metals) contaminants in food cannot be estimated indirectly because their level is not determined by a technical functionality in the food itself like food additives or in the raw commodity like pesticides or veterinary drugs. Furthermore, the concentration of chemical contaminant can decrease or increase during storage and processing. Therefore analytical measurements are necessary to establish the concentration level(s) to be combined with food consumption data in order to assess the dietary exposure. The results of analytical measurements will follow a distribution depending on the nature of the contaminant but also on where, when and how (e.g. targeted or random sampling) the samples were collected.

For a contaminant with a long-term toxicity, concentrations are generally estimated in 2 different ways:

• The average measured concentration can be used to represent the long-term dietary exposure, assuming that a consumer is unlikely to consume regularly highly contaminated food. Available data can have been obtained both from single samples and from pooled samples and the mean can be weighted for pooled samples by the number of initial samples regrouped before the chemical analysis. Non-detects and unquantified results may be dealt with in various ways including assumed zero, assumed equal to the limit value or half the limit value, assumed to be distributed uniformly between zero and the limit value, or extrapolating a distribution from data above the limit value.

• The full distribution of contaminant concentrations can be used in a probabilistic modelling of the dietary exposure. In that case, the uncertainty is related to the treatment of non-detects and unquantifiable data and to the precision of the tails of the distribution.

For food consumption data, also two different approaches may be taken:

• Exposure is calculated for the 'average' consumer, those with average consumption of foodstuffs, and for the 'high' consumer, those with e.g. 95th percentile consumption using the EFSA's Comprehensive European Food Consumption Database) food consumption database. If such data is not available, consumption scenarios are used.

• The full distribution of food consumption data is used in a probabilistic modeling.

In many cases the main uncertainty in exposure assessment of chemical contaminants is related to the treatment of non-detects and unquantifiable data as for quite a number of contaminants the number of non-detects is 60 to 80%. EFSA has published an opinion on how to deal with this in March 2010 (EFSA, 2010c).

For a contaminant with a short-term mechanism of toxicity (e.g. some marine biotoxins), the highest concentration recorded in a portion of foodstuff is often used to estimate the consumer exposure. These can be useful in some cases but are not the most adequate data as they may underestimate concentration peaks. Besides the fact that highest concentration recorded in a portion of foodstuff is often used to estimate the consumer exposure, a maximal portion size is often assumed (e.g. 400g for shellfish).

In the context of possibly applying the TTC concept to a compound of unknown toxicity, it is very unlikely that the data available will allow for a probabilistic modeling of exposure. Therefore, in most cases, human exposure will be calculated by multiplying average measured concentration by food consumption estimates for 'high' (and 'average') consumers.

FEEDAP – Panel on Additives and Products or Substances used in Animal Feed

Two main issues characterise consumer exposure assessment for substances used in animal feed.

Dietary exposure is restricted to specific foods of animal origin

In particular, since the mission is to assess the safety of intended use of feed additives that can carry over into the human diet, only those foods relevant to such intended use are considered. For instance, if a compound is not intended for use in laying birds, deposition in eggs is not normally relevant to consumer exposure. However, deposition in eggs, hence consumer exposure, might result from inadvertent contamination of feed chains, as has occurred with several coccidiostats authorized for use in feeds of chickens for fattening but not laying hens. Such cases have been assessed by the CONTAM Panel, as undesirable substances.

Dietary exposure of consumers is mediated by the metabolism of the target farm animal species

Consumer exposure assessment depends on:

a) pharmacokinetic studies, identifying whether the parent compound or one or more metabolite are the most representative residue, and

b) deposition studies, where the deposition of the additive in edible tissues and products is assessed in field conditions, for time length compatible with animal production and at the maximum levels intended for use.

When required, such as in the case of substances that are not normally present in the body, the above studies lead to the identification of marker residue(s) (i.e., biologically significant and in known proportion to total residues) and of maximum residue limits (MRL, based on marker residue, aimed at keeping the exposure below the ADI).
However, in many cases, no such parameters are needed. In particular no need for MRL or marker residue is normally foreseen for:

- biological feed additives (enzymes, probiotics) that normally do not give residues, or
- natural diet components used as nutritional additives (trace elements, vitamins) where consumer exposure assessment is based on the additional intake provided by the use of the substance as feed additive, compared to background dietary intake, and the likelihood that the resulting total intake would be higher than the tolerable upper intake level (UL) as defined in human nutrition.

Consumer exposure is assessed in a conservative way, according to a Theoretical Maximum Daily Intake approach, based on the daily consumption of foods containing the additive at MRL and with a food basket featuring high, as well as highly conservative, levels of daily consumption. The following standard food basket is currently used by the Panel for theoretical maximum daily intake for substances that are not normally present in the body:

- meat (mammals/birds): 300 g, or fish flesh (including skin in natural proportion): 300 g fat or chicken's fat (including skin in natural proportion): 50 g; liver: 100 g; kidney: 50 g (10 g for substances intended for used in birds only, such as coccidiostats);
- egg: 100 g; milk: 1500 g; honey: 20 g (never applied till now by FEEDAP).

Of course adjustments must be made depending on the intended use (e.g., milk will not be considered for a feed additive not intended for use in dairy animals). On a case-by-case basis, other sources of exposure resulting from other uses of the same additive are considered; thus, the MRL should keep the exposure below a fraction of the ADI (e.g. 50%).

Whereas this highly conservative approach is appropriate for substances that are not normally present in the body, the FEEDAP Panel uses a different approach towards nutrients (vitamins, trace elements). In such cases, exposure assessment should consider:

- reliable EU data on background dietary intake, possibly allowing to distinguish the fraction attributable to foods of animal origin as well as the high intake levels, and
- reliable EU data on the realistic consumption of relevant foods of animal origin, possibly allowing identification of levels in high consumers and in children.

User/worker exposure

In addition to consumer exposure, the FEEDAP ppanel also has to estimate exposure of user/workers through inhalation and dermal route. The dusting potential and the particle size distribution of the additive are key parameters to develop exposure estimates. When exposure may occur, worst case scenario compatible with the intended use(s) of the additives is developed (EFSA, 2010f).

PPR – Panel on Plant Protection Products and their residues

Assessment of exposure to a plant protection product via the diet is almost always substance-specific, i.e. generic scenarios are not used. Such exposure assessment is based on knowledge of the actual or predicted concentrations of the pesticide in foodstuffs and the amount of the foodstuffs consumed. To date, most assessments have been based on deterministic approaches, although increasingly probabilistic approaches are being introduced. For the calculation of the expected exposure using deterministic methodologies, concentrations of the pesticide in foodstuffs for a new active are predicted on the basis of field trials in which the substance is applied according to good agricultural practice (GAP), taking into consideration the rate and number of applications of the active, the method of application and any pre-harvest interval. Parameters are maximised within those possible to achieve plausible worst case values. For chronic assessments, the supervised trials median residue (STMR) level is now used. Information on food consumption can be obtained in a number of ways, for instance by using data provided by MS for the development of the EFSA PRIMo (EFSA Pesticide Residue Intake Model). In future, these data will be replaced with the data provided to EFSA in the framework of the EFSA comprehensive European Food consumption data. A wide use was made of the EFSA Concise European Food Consumption Database (EFSA, 2008q). This is now being expanded, to produce EFSA's Comprehensive European Food Consumption Database (EFSA, 2011b)

Dietary exposure is usually calculated for the 'average' consumer, those with average consumption of foodstuffs, and for the 'high' consumer, those with 95th percentile consumption. In determining exposure a number of issues have to be considered. These include, when using monitoring data on a pesticide, how values at the limit of reporting will be treated; possible changes in pesticide concentration with processing of the foodstuff; carry-over of pesticide into following crops or into meat and dairy products through animal feed.

Similar considerations apply to metabolites of potential toxicological relevance. In this case, detailed information on the pattern and distribution of metabolites in foodstuffs is required.

EFSA also has to estimate exposure of operators, workers, residents and bystanders. In these cases, in addition to exposure by the oral route, consideration has to be given to exposure by the dermal and inhalation routes. Estimates are obtained using a combination of experimental data, for example for dermal absorption and appropriate models, for example the EUROPOEM Predictive Operator Exposure Model. The EFSA PPR Panel has developed draft updated guidance on the assessment of dermal absorption and an opinion on the science behind the draft guidance (see http://www.efsa.europa.eu/en/scdocs/scdoc/52e.htm). The PPR Panel has recently published draft guidance on the assessment of exposure of operators, workers, residents and bystanders to pesticides and an opinion on the science behind the draft guidance (see http://www.efsa.europa.eu/en/scdocs/scdoc/1501.htm).

APPENDIX F

Does a TTC value of 0.15 µg/day provide a sufficient margin also for heritable/mutagenic effects?

The dimension of the genetic risk associated with exposure to genotoxic substances at the TTC value of 0.15 µg/day (equivalent to 0.0025 µg/kg bw per day) can, in principle, be estimated using quantitative data on chemically-induced heritable effects. However, data amenable for the quantitative evaluation of genetic risk are only available for a very limited set of substances, and it is expected that no further data will be produced as relevant *in vivo* test methods use large numbers of animals. Most available data concern four substances selected for an EC/US exercise on comparative genetic risk assessment (Waters & Nolan, 1995): the industrial chemicals acrylamide, 1,3-butadiene and ethylene oxide, and the cancer chemotherapeutic agent cyclophosphamide. Other quantitative data on heritable effects concern the ethylating agents ethyl methanesulphonate and ethylnitrosourea, selected for a molecular dosimetry comparative study, and the chemotherapeutic drug procarbazine.

A selection of test results, as reported by the authors, on transmissible effects induced by these chemicals in male mice is summarised in Table 1 below. Mutation frequencies were estimated using two different approaches, i.e. the Direct Method and the Doubling Dose (or Indirect) Method (Ehling, 1988). Briefly, the Direct Method extrapolates the expected overall genetic burden in humans from the observed dominant mutation rate per locus in mice, multiplied by the number of loci in humans at which dominant mutations occur. The second approach avoids a specific estimate of the number of human loci involved in deleterious dominant mutations, but requires an estimate of the overall spontaneous mutation frequency in humans to dominant alleles. The main findings are described below.

Acrylamide

Acrylamide affected several stages of mouse spermatogenesis. Specific-locus mutations were induced both in spermatogonia and post-meiotic stages (spermatozoa and late spermatids). Chromosomal effects (dominant lethals and heritable translocations) were mainly induced in later stages (spermatids and early spermatozoa). Doubling Doses (DD) range from 53 mg/kg bw, when estimated by the specific-locus test, to 0.39 mg/kg bw, when estimated with the heritable translocation test. Based on these findings, the frequency of dominant genetic disease burden in the offspring of males exposed to the limit concentration of acrylamide in drinking water (0.5 µg/L, corresponding to 1.3×10^{-5} mg/kg bw for a 75 kg person drinking 2 L of water) was calculated. The number of induced genetic diseases per million offspring ranged from 7.3×10^{-5} to 3.0×10^{-2} (Dearfield et al., 1995). Approximately 6-fold lower incidences can be calculated for the daily intake of acrylamide at the TTC level of 0.15 µg/day.

Cyclophosphamide

Post-meiotic cell stages are most sensitive to the genotoxic effects of cyclophosphamide. DD in the mouse morphological specific-locus test were 4 and 16 mg/kg bw for treatment of post-meiotic cells, while no detectable increase in mutant frequency was observed with treatment of spermatogonial stem cells. It must be noted that the above figures are based on a low number of observations (mutants in progeny), and thus are highly uncertain. However, based on the DD of 4 mg/kg bw it was calculated that the excess incidence of dominant and X-linked diseases for the acute exposure at 1 mg/kg bw would be 625 affected individuals per million liveborn (Anderson et al., 1995). Extrapolated to the TTC exposure level, such an estimate is approximately 2×10^{-3} additional cases per million of offspring.

Ethylene oxide

The frequency of recessive mutations induced in mouse spermatogonia following inhalational exposure to ethylene oxide was calculated to be approximately 0.2 to 2×10^{-6} for an inhalational exposure of 1000 ppm for an hour (Natarajan et al., 1995). Considering the ventilation rate of the mouse, the concentration x time value (1000 ppm/h) can tentatively be converted into a weight-to-

weight figure (135 mg/kg bw). The corresponding incremental risk of recessive mutations for an exposure at the TTC level can be calculated by linear extrapolation, and is approximately 3 x 10^{-15}. The incremental risk of dominant visible mutations was estimated to be about 1.3 x 10^{-5} at 1000 ppm/h, which corresponds to ~ 2.5 x 10^{-13} at the TTC exposure level.

Ethylnitrosourea, ethylmethansulphonate and procarbazine

Mutation frequencies after spermatogonial treatments were determined in the offspring of mice using different genetic end-points, involving different numbers of loci (Ehling, 1988, Ehling & Neuhäuser-Klaus, 1989). Based on figures shown in Table 1, the induced mutation frequencies for treatment with 1 mg/kg bw of ethylnitrosourea and procarbazine range from 3.3 x 10^{-6} to 5 x 10^{-7} and from 1 x 10^{-7} to 0.5 x 10^{-8}, respectively. Approximately 4 x 10^{5}-fold lower frequencies are obtained when extrapolated to the TTC exposure level. Also for ethylmethansulphonate, a very small incremental risk is associated with exposure at the TTC level, given that such an exposure level is approximately 10^{8}-fold lower than the experimentally determined doubling dose (175 mg/kg bw).

Table 1. Estimated germ cell mutation frequencies in mice

Substance	Test system	Germ cell mutation frequency		Reference
		Induced mutation Frequency	Doubling Dose	
Acrylamide	Mouse specific-locus test[a]		53 mg/kg bw	Ehling & Neuhäuser-Klaus, 1992
	Mouse heritable translocations		1.8 mg/kg bw 3.3 mg/kg bw 0.39mg/kg bw	Shelby et al, 1987; Adler et al, 1994 Adler et al, 1990
Cyclophosphamide	Mouse specific-locus test[a]		4 mg/kg bw[b] 16 mg/kg bw[c]	Ehling & Neuhäuser-Klaus, 1988
Ethylene oxide	Mouse specific-locus test[a,d]	0.21 ± 0.28 x 10^{-6}/1000 ppm h		Russell et al., 1984
	Mouse specific-locus test[e]	1.3 x 10^{-5}/1000 ppm h		Lewis et al., 1986
Ethyl methane sulphonate	Mouse specific-locus test[a]		175 mg/kg bw	Ehling & Neuhäuser-Klaus, 1989
Ethylnitrosourea	Mouse specific-locus test[a]	5.7 x10^{-4} at 160 mg/kg bw		Ehling, 1988
	Mouse specific-locus test[e,f]	7.3 x 10^{-5} at 160 mg/kg bw		
Procarbazine	Mouse specific-locus test[a]	4.4 x 10^{-5} at 600 mg/kg bw		Ehling, 1988
	Mouse specific-locus test[e,f]	0.3 x 10^{-5} at 600 mg/kg bw		

[a] specific-locus visible recessive mutations (7 loci)
[b] treatment of late spermatids and spermatozoa
[c] treatment of differentiating spermatogonia and spermatids
[d] treatment of spermatogonia
[e] dominant visible mutations
[f] dominant cataract mutations (30 loci)

Thus, even taking into account the extremely limited database, and additional uncertainties related to the route of exposure, stage-related variation in sensitivity of germ cells, the lack of data on female germ cells, and the possible accumulation of genetic damage in pr-meiotic cells during chronic exposure, the available data on chemically induced transmissible effects suggest that the incremental risk associated with genotoxic chemical exposure at the proposed TTC exposure level is extremely low, if any. Based on the available data, when applied to a genotoxic agent the TTC value of 0.15 µg/day (0.0025 µg/kg bw per day) could also cover transmissible effects, beyond cancer.

This conclusion could be anticipated to some extent in view of the apparent relative lower sensitivity of germ cells compared to somatic ones. Many studies have addressed the relationship between somatic and germ cell mutations, reaching the similar conclusion that there is still no evidence of germ line specific mutagens, and that when a mutagenic response is elicited in germ cells, an even greater response is typically detected in somatic cells. This fact is considered to be attributable to the different chemical accessibility of somatic versus germ cells, rather than to intrinsic differences in the ability to process pre-mutagenic lesions, as demonstrated by comparative molecular dosimetry studies (Van Zeeland et al., 1985). The possibility for a systemically available substance to reach gonadal targets is largely modulated by pharmacokinetic and anatomic factors, including the compartimentalisation of gonads. The Sertoli cell barrier, in particular, is believed to play a significant role in protecting meiotic and post-meiotic male germ cells, limiting the access of exogenous chemicals to gonads (Russell, 1990).

For references, see list in main text.

Abbreviations:

AHAW: Scientific Panel on Animal Health and Animal Welfare
ADI: Acceptable daily Intake
AChE: Anti-cholinesterase
ANS: Panel on Food Additives and Nutrient Sources added to food
ARfD: Acute Reference Dose
Biohaz: Scientific Panel on Biological Hazards
CEF: Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CONTAM: Scientific Panel on Contaminants in the Food Chain
CPDB: Carcinogenicity Potency Database
DART: Developmental and Reproductive Toxicology database
DSSTox: Distributed Structure-Searchable Toxicity Database
EC: European Commission
ECHA: European CHemicals Agency
EPA: Environmental Protection Agency
FDA: Food and Drug Administration
FEEDAP: Scientific Panel on Additives and Products or Substances used in Animal Feed
GMO: Scientific Panel on Genetically Modified Organisms
JRC: Joint Research Centre
JECFA: Joint FAO/WHO Committee on Food Additives
JMPR: Joint FAO/WHO Meetings on Pesticides Residues
IGHRC: Inter-departimental Group of Health Risk from Chemicals
IRIS: Integrated Risk Information System
LOEL: Low Observed Effect Level
NDA: Scientific Panel on Dietetic Products, Nutrition and Allergies
NOEL: No Observed Effect level
OECD: Organisation for Economic Co-operation and Development OP: Organophosphate
PCA: Principal Component Analysis
PLH: Scientific Panel on Plant Health
PLS: Partial least Squares
PPR: Scientific Panel on Plant Protection Products and their Residues
QSAR: Quantitative Structure Activity Relationship
SCCP: Scientific Committee on Consumer Products
SCF: Scientific Committee on Foods
SIMCA: Soft Indipendent Modeling of Class Analogy
TD: Tolerable Dose
TTC: Threshold of Toxicological Concern
WHO: World Health Organization