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Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet

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Abstract

The threshold of toxicological concern (TTC) is a pragmatic risk assessment tool that is based on the principle of establishing a human exposure threshold value for all chemicals, below which there is a very low probability of an appreciable risk to human health. The concept that there are levels of exposure that do not cause adverse effects is inherent in setting acceptable daily intakes (ADIs) for chemicals with known toxicological profiles. The TTC principle extends this concept by proposing that a de minimis value can be identified for many chemicals, in the absence of a full toxicity database, based on their chemical structures and the known toxicity of chemicals which share similar structural characteristics. The establishment and application of widely accepted TTC values would benefit consumers, industry and regulators. By avoiding unnecessary toxicity testing and safety evaluations when human intakes are below such a threshold, application of the TTC approach would focus limited resources of time, cost, animal use and expertise on the testing and evaluation of substances with the greatest potential to pose risks to human health and thereby contribute to a reduction in the use of animals. An Expert Group of the European branch of the International Life Sciences Institute—ILSI Europe—has examined the TTC principle for its wider applicability in food safety evaluation. The Expert Group examined metabolism and accumulation, structural alerts, endocrine disrupting chemicals and specific endpoints, such as neurotoxicity, teratogenicity, developmental toxicity, allergenicity and immunotoxicity, and determined whether such properties or endpoints had to be taken into consideration specifically in a step-wise approach. The Expert Group concluded that the TTC principle can be applied for low concentrations in food of chemicals that lack toxicity data, provided that there is a sound intake estimate. The use of a decision tree to apply the TTC principle is proposed, and this paper describes the step-wise process in detail. Proteins, heavy metals and polyhalogenated-dibenzodioxins and related compounds were excluded from this approach. When assessing a chemical, a review of prior knowledge and context of use should always precede the use of the TTC decision tree. The initial step is the identification and evaluation of possible genotoxic and/or high potency carcinogens. Following this step, non-genotoxic substances are evaluated in a sequence of steps related to the concerns that would be associated with increasing intakes. For organophosphates a TTC of 18μg per person per day (0.3 μg/kg bw/day) is proposed, and when the compound is not an OP, the TTC values for the Cramer structural classes III, II and I, with their respective TTC levels (e.g. 1800, 540 and 90 μg per person per day; or 30, 9 and 1.5 μg/kg bw /day), would be applied sequentially. All other endpoints or properties were shown to have a distribution

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of no observed effect levels (NOELs) similar to the distribution of NOELs for general toxicity endpoints in Cramer classes I, II and III. The document was discussed with a wider audience during a workshop held in March 2003 (see list of workshop participants).

Keywords: Risk assessment; Threshold of toxicological concern (TTC); Carcinogenicity; Neurotoxicity; Teratogenicity; Exposure; de Minimis Risk; Toxicity; Food safety

1. Introduction

The threshold of toxicological concern (TTC) is a principle, which refers to the establishment of a human exposure threshold value for all chemicals, below which there would be no appreciable risk to human health. The concept that exposure thresholds, or safe levels of exposure, can be identified for individual chemicals in the diet, is already widely embodied in the practice of regulatory bodies in setting acceptable daily intakes (ADIs) for chemicals with known toxicological profiles. However, the TTC concept goes further than this in proposing that a de minimis value can be identified for many chemicals, including those of unknown toxicity, based on consideration of their chemical structures. The de minimis concept accepts that human exposure threshold levels exist for different types of chemicals/structures. Uncertainties are an inherent part of the risk characterisation of chemicals, even when there is a full toxicological database. For example uncertainties normally exist in relation to the sensitivity of the test species studied compared to humans, and the validity of the test methods to detect all adverse effects relevant to humans. The TTC can be used to assess the likelihood that a particular level of exposure to a chemical would be without toxic effects in the absence of chemical-specific toxicity data, based on the available toxicity data for a wide range of chemicals; in other words knowledge from the "world of chemicals" is balanced against very low levels of intake of the chemical under evaluation. Reviews of available data on the "world of chemicals" are used to establish threshold levels of exposure, related to the chemical structure, which would be without significant risk. Exposure below the relevant threshold level would pose no appreciable risk to human health, despite the absence of toxicity data on the compound being considered.

The establishment of more widely accepted TTC values would benefit consumers, industry and regulators. In avoiding unnecessary extensive toxicity testing and safety evaluations when human intakes are below such a threshold, it would focus limited resources of time, animal use, cost and expertise on the testing and evaluation of those substances with greater potential to pose risks to human health, and would contribute to a reduction in the use of animals for safety testing. Application of the TTC principle would not only be used for priority setting for toxicity testing, but could also be used to indicate analytical data needs and to set priorities for levels of "inherent concern". It is considered to be a preliminary step in safety assessment.

The concept of a TTC evolved from the review by Munro (1990) of the Threshold of Regulation in the USA, and further developments by Munro et al. (1996) based on analysis of the chronic toxicity data of chemicals in three structural classes identified according to the Cramer et al. (1978) decision tree. The "TTC concept" formed the scientific basis of the US Food and Drug Administration Threshold of Regulation for indirect food additives (Federal Register, 1993; see also Food and Drug Administration, 1983, 1993). The TTC principle has also been adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its evaluations of flavouring substances (JECFA, 1993, 1995, 1999; Munro et al., 1996, 1999): since 1996 a decision tree incorporating different TTCs related to structural class has been used for the safety evaluation of over 1200 flavouring substances.

A threshold of regulation is used by the US Food and Drug Administration to review components of food contact materials with low exposures and relates to a dietary concentration giving an intake of 1.5 μg per person per day (0.025 μg/kg bw/day). Below this level FDA requires no specific toxicity testing and performs only an abbreviated safety assessment, mainly focussed on intake assessment.

The thresholds used in the TTC approach are intakes, expressed in μg per person per day, below which a given compound of known structure is not expected to present a toxicological concern. Therefore, the TTC for a given compound has to be compared with an estimate of human exposure to this chemical to determine whether or not there is a safety concern, and whether or not more detailed chemical-specific toxicity data are necessary. Thus, an appropriate human exposure estimate is a necessity for applying the TTC principle. The TTCs are calculated assuming a body weight of 60 kg, and this may need to be taken into account when the intake estimates for a compound are considered in relation to the relevant TTC (see below).

An Expert Group of the Threshold of Toxicological Concern Task Force of the European branch of the International Life Sciences Institute (ILSI Europe) has examined the TTC principle, which was based on general toxicity endpoints (including carcinogenicity), in relation to its applicability in food safety evaluation. The

application of the TTC concept in food safety evaluation is not meant to replace other regulatory procedures but rather is a preliminary step in the risk assessment process to aid in the assessment of whether chemical-specific toxicity data are necessary.

In an earlier publication (Kroes et al., 2000) the TTC principle was examined for general toxicity endpoints (including carcinogenicity) as well as for specific endpoints, namely neurotoxicity and developmental neurotoxicity, developmental toxicity and immunotoxicity. It was shown that the cumulative distributions of the no-observed-effect-levels (NOELs—equivalent in meaning to a no-observed-adverse-effect-level or NOAEL) for developmental toxicity did not differ greatly from the cumulative distribution of NOELs for chronic toxicity of class III chemicals as described by Munro et al. (1996). The NOELs for immunotoxicity did not differ from the NOELs for other endpoints. In the case of neurotoxicants, the distribution was almost one order of magnitude lower than the distribution of NOELs for chronic toxicity of class III chemicals. The distribution of the NOELs of class III chemicals differed considerably (by about three orders of magnitude higher) from the distribution of 10^{-6} risk levels derived by linearised low-dose extrapolation for the carcinogens contained in the Gold database (Gold et al., 1989, 1990, 1991, 1993, 1995, 1999; Gold and Zeiger, 1997).

The present paper reports the considerations of the ILSI Europe Expert Group related to a number of further questions regarding the application of the TTC principle. Consideration was given to providing increased safety assurance by the identification of structural alerts for high potency carcinogens, and to the question of whether neurotoxicants or teratogens should be considered as separate classes. In addition further consideration was given to endocrine disrupting chemicals and how food allergies, hypersensitivity reactions and intolerances should be considered in relation to the application of the TTC principle. Finally, the Expert Group evaluated whether separate consideration of metabolism and accumulation was necessary in the application of a TTC.

Based on the deliberations of the ILSI Europe Expert Group, and a Workshop held in March 2003 [see list of workshop participants (Appendix A)], a decision tree incorporating a tiered approach was developed as guidance on how and when the TTC principle could be applied as a preliminary step in food safety evaluation.

2. Consideration of structural alerts for high potency carcinogenicity

Cheeseman et al. (1999) have considered the application of structural alerts for carcinogenicity (as defined by Ashby and Tennant, 1991) to increase safety assurance in FDA's Threshold of Regulation procedure, and to establish a series of thresholds by the progressive elimination from regulatory consideration of compounds in structural classes of highest concern. In order to identify the structural groups of most concern at the lowest dietary concentrations, the scheme of structural alerts proposed by Ashby and Tennant (1991) and by Cheeseman et al. (1999) was examined. The 709 compounds extracted by Cheeseman et al. (1999) from the Gold carcinogenic potency database (Gold and Zeiger, 1997), plus additional compounds to give a total of 730 compounds, were classified (Table 1). Some structural groups remained unchanged from the analysis of Cheeseman et al. (1999) including N-nitroso compounds, alpha-nitro–furyl compounds, organophosphorous compounds, and compounds containing heavy metals. Several structural groups proposed by Cheeseman et al. (1999) were reconsidered and divided into smaller more homogeneous structural groups. These subdivided structural groups included (1) the endocrine disruptor structural group which was divided into four groups (i.e., steroids, highly chlorinated compounds, organotin compounds, and tetrahalogenated-(2,3,7,8) dibenzodioxins and related compounds); (2) the group of "hydrazines, triazenes, azides, azo, and azoxy compounds" which was divided into separate groups of hydrazines, azoxy compounds, and azo compounds; and (3) the group of strained heteronuclear rings which was subdivided into a group of compounds containing strained rings and three other groups of compounds known to produce metabolic products containing strained rings (i.e., aflatoxin-like compounds, polycyclic aromatic hydrocarbons, and vinyl containing compounds). Additional structural groups were identified based on alerts identified by Ashby and Tennant (1991). These include aromatic amines and aromatic nitro compounds. Finally, the remaining compounds in the database alerted by Ashby's and Tennant's scheme were combined into a group of "miscellaneous Ashby alerts." This last group of compounds includes most of what Ashby and Tennant classified as alkylating agents. Compounds with more than one structural alert were incorporated into the structural group with the higher/highest potency. The results of this analysis are presented in Table 1, which shows the number and the fraction of compounds in each structural group that would result in an upper bound risk for cancer of greater than one in one million (calculated by linear extrapolation from the TD50) for different intakes expressed in μg/person/day. The conservatism built into this approach is discussed below.

The analysis of the expanded database of 730 compounds focused on identifying the structural alerts that would give the highest calculated risks if present at very low concentrations in the diet. The differences between

the different structural alerts was most apparent in the data for the fraction of compounds within each group giving an estimated upper bound risk of cancer of greater than one in one million, when present in the diet at a concentration of 0.15 μg per person per day (0.0025 μg/kg bw/day) (Table 1). It should be emphasized that any group of compounds containing alerts for carcinogenicity should be of concern in the safety review of substances entering the food supply. However, some structural groups were identified to be of such high potency that if a TTC were to be established it would need to be set at a much lower dietary concentration than a TTC for other structural groups. Five groups had a significant fraction of their members that may still be of concern at an intake of 0.15 μg per person per day (0.0025 μg/kg bw/day) (Table 1). The five structural groups are aflatoxin-like compounds, N-nitroso-compounds, azoxy-compounds, steroids, and polyhalogenateddibenzo-*p*-dioxins and-dibenzofurans and these were termed the ''Cohort of Concern'' or COC. Steroids and polyhalogenated dibenzo-*p*-dioxins and-dibenzofurans are considered to be non-genotoxic carcinogens which would show a threshold in their dose-response relationships: therefore the calculation of theoretical upper bound risks using linearised models based on animal carcinogenicity bioassays is unrealistic and irrelevant, and would not be used if the risk assessment were to be based on compound-specific data. Thus, the COC for high potency genotoxic carcinogens comprises aflatoxin-like compounds, N-nitroso-compounds and azoxy-compounds.

The incorporation of these considerations of genotoxicity and carcinogenicity into a decision tree is discussed below. It is suggested that a TTC would not be appropriate for chemicals with the structural alerts for high potency carcinogenicity. A TTC is proposed for compounds with other structural alerts for carcinogenicity, using a highly conservative approach based on linear extrapolation of the animal dose–response data down to a theoretical risk of one in a million.

2.1. Consideration of neurotoxicants as a separate class

In the previous ILSI Europe paper on TTC (Kroes et al., 2000), it was observed that the cumulative distribution of NOELs for neurotoxic compounds differed from the distribution of NOELs for chronic toxicity for structural class III (Cramer et al., 1978).

The database used by Kroes et al. (2000) was considered to be biased toward high potency because the majority of the compounds were organophosphates (OPs) (25 out of 45) which are designed to be potent neurotoxicants. Amongst the 20 most potent compounds (NOEL <1 mg/kg/day) of the database, 16 were OPs. Because of this bias towards OP compounds, that are developed specifically for their neurotoxic

Table 1
Numbers and fractions of compounds in different structural groups that are estimated to give a risk greater than one in a million at different intake levels

Structural Group	0.15 mcg		1.5 mcg		3 mcg		6 mcg		15 mcg		30 mcg		60 mcg		150 mcg		Total
	n	*F*	*n*	*F*	*n*	*F*	*n*	*F*	*n*	*F*	*n*	*F*	*n*	*F*	*n*	*F*	
Aflatoxin-like compounds	**5**	**1**															**5**
Aromatic amines	5	0.03	51	0.31	71	0.44	82	0.51	106	0.65	126	0.78	138	0.85	153	0.94	162
Aromatic nitrates	2	0.06	8	0.24	12	0.36	12	0.36	15	0.45	21	0.64	24	0.73	30	0.91	33
Azo compounds	0	0	9	0.50	9	0.50	10	0.56	12	0.67	14	0.78	16	0.89	17	0.94	18
Azoxy compounds	**4**	**0.80**	**4**	**0.80**	**4**	**0.80**	**5**	**1**									**5**
Benzidine derivatives	2	0.14	6	0.43	8	0.57	9	0.64	10	0.71	12	0.86	12	0.86	13	0.93	14
Carbamates	0	0	8	0.40	8	0.40	10	0.50	15	0.75	17	0.85	18	0.9	19	0.95	20
Heavy metal containing compounds	1	0.14	4	0.57	4	0.57	5	0.71	6	0.86	7	1					7
Highly chlorinated compounds	5	0.09	23	0.43	27	0.50	30	0.56	35	0.65	42	0.78	43	0.8	51	0.94	54
Hydrazines	2	0.04	30	0.53	35	0.61	37	0.65	47	0.82	47	0.82	52	0.91	56	0.98	57
Miscellaneous ashby alerts	2	0.05	5	0.12	8	0.2	13	0.32	25	0.61	32	0.78	34	0.83	36	0.88	41
α-Nitro Furyl Compounds	1	0.03	16	0.47	24	0.71	31	0.91	33	0.97	34	1					34
N-Nitroso Compounds	**47**	**0.45**	**90**	**0.86**	**96**	**0.91**	**99**	**0.94**	**102**	**0.97**	**105**	**1**					**105**
Organophosphorus compounds	0	0	5	0.29	5	0.29	6	0.35	8	0.47	11	0.65	14	0.82	15	0.88	17
Steroids	5	0.45	9	0.82	9	0.82	10	0.91	10	0.91	11	1					11
Strained rings	1	0.07	9	0.60	11	0.73	11	0.73	12	0.8	13	0.87	14	0.93	15	1	15
Tetrahalogenated dibenzodioxins and dibenzofurans (2,3,7,8)	2	0.40	2	0.40	2	0.40	2	0.40	3	0.6	3	0.6	3	0.6	3	0.6	5
Vinyl containing compounds	2	0.05	13	0.33	16	0.40	22	0.55	28	0.7	34	0.85	38	0.95	40	1	40

Absolute numbers of compounds (*n*) in various structural groups in the database that would give estimated risks greater than 1 in 10^6 if the intake were at values given in the column heading (calculated for a 60 kg person and an intake of 3 kg of diet per day) along with the fraction (*F*) of all members of each structural group. Compounds excluded at an early stage of the decision tree (Fig. 2; Steps 1 and 3) are shown in bold font. The risk estimates are based on linearised low-dose extrapolation, which would not be appropriate for compounds such as steroids and TCDD which act via non-genotoxic mechanisms.

actions, the neurotoxicity database would over-predict the potency that would be seen in non-selected (non-OP) compounds from within the "world of chemicals" that produce neurotoxicity. In addition, the database was conservative because the parameters selected for the OPs were particularly sensitive (cholinesterase inhibition) and in certain cases were not necessarily associated with a functional deficit (e.g. plasma cholinesterase). An analysis was undertaken to determine if replacement of these endpoints by parameters of greater neurotoxicological relevance would reduce the difference found between the distributions of NOELs for neurotoxicity and for structural class III (Munro et al., 1996) to an insignificant level. In such a case, the identification of neurotoxicity structural alerts would not be necessary in the TTC-Decision Tree.

Four different endpoints were considered in the construction of the database: (1) neurobehavioural alterations, (2) brain cholinesterase (ChE) inhibition, (3) red blood cell (RBC) ChE inhibition and (4) plasma ChE inhibition. Neurobehavioural alterations and brain ChE inhibition are parameters clearly related to neurotoxicity. RBC ChE is the same enzyme as the brain ChE and therefore its inhibition is considered of neurotoxicological relevance (Lotti, 1995; Padilla 1995; Chen et al., 1999). Plasma ChE is not related to brain ChE and its inhibition is usually considered as a biomarker of exposure with little neurotoxicological relevance (Lotti, 1995; Padilla 1995; Chen et al., 1999). A review of the original studies used to construct the database revealed that plasma ChE inhibition was the parameter selected for four OPs (azinphosmethyl, ethion, pirimiphosmethyl and quinalphos). The effect of replacement of the plasma ChE-inhibition endpoints by parameters of neurotoxicological relevance on the NOEL was investigated. JMPR monographs from the last 10 years were reviewed [Joint FAO/WHO Meeting on Pesticide Residues (JMPR) 1990a,b,c, 1992a,b, 1993a,b,c,d, 1994a,b,c,d, 1995a,b,c, 1996, 1997a,b, 1999]. Chronic toxicity studies of 20 OPs were evaluated for the three ChE inhibition endpoints using 20% inhibition as a threshold for significance. Based on this review, no clear relationship was found between brain, RBC and plasma ChE inhibition. For the four OPs in the database characterized by plasma ChE-inhibition (azinphosmethyl, ethion, pirimiphosmethyl, quinalphos) the literature was scanned for other studies and the impact of replacing the existing NOELs based on plasma ChE inhibition by other more relevant NOELs was investigated; however, inhibition of plasma, erythrocyte and brain ChE occurred at similar doses. In addition it was recognized that in some cases, dog studies, which were not included in the database of Kroes et al., (2000), may be more sensitive than the rodent studies used to construct the database, indicating that the database is not always over-conservative. It was therefore concluded that a step assessing structural alerts for neurotoxicity should be included in a TTC Decision Tree.

The majority of the most potent compounds in the neurotoxicity database are OPs, and the cumulative distribution of NOELs of OPs differed by one order of magnitude from the distribution of NOELs of neurotoxicants that are not OPs (Fig. 1). The Expert Group concluded that the introduction of a step to identify OP structural alerts instead of neurotoxicity alerts into the decision tree would give a selective step for the most potent neurotoxicants. An analysis of the distribution of NOELs of 31 OPs revealed a 5th percentile value lower than the 5th percentile NOEL of the class III compounds of the Munro database. In the previous ILSI Europe-work (Kroes et al., 2000) a TTC for general neurotoxicants was not derived because the database was considered not to be sufficiently robust, but a possible TTC for neurotoxicity (based on the 5th percentile NOEL for chronic and subchronic studies divided by an uncertainty factor of 100 and assuming a body weight of 60 kg) was identified at 18–24 μg per person per day (0.3–0.4 μg/kg bw/day). Munro et al. (1999) analysed 31 OPs (using ChE-inhibition as an endpoint), and derived a TTC (based on the 5th percentile of NOELs divided by 100 and corrected for body weight) of 18 μg per person per day (0.3 μg/kg bw/day).

Together, these findings demonstrate the need for the introduction of a separate step based on organophosphate structural alerts into a TTC Decision Tree. Since the slight differences in TTCs in the different analyses are most likely driven by the OPs in the database, the

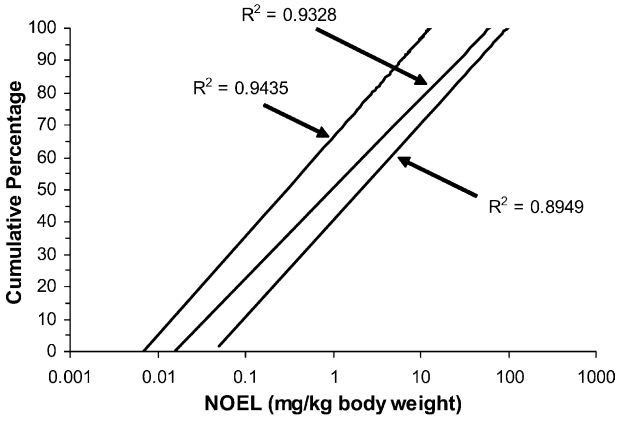

Fig. 1. Cumulative distributions of the NOELs for neurotoxicity. OP compounds ($n = 19$) are shown as the logarithmic regression to the left ($r^2 = 0.9435$), all compounds in the database ($n = 45$) are shown as the middle logarithmic regression ($r^2 = 0.9328$), and non-OP compounds ($n = 26$) are shown as the logarithmic regression to the right ($r^2 = 0.8949$). NOEL values were not available for 5 non-OP compounds and in these cases the reported LOEL (lowest observed effect level) was divided by an uncertainty factor of 3 to predict the likely NOEL. Applying an uncertainty factor of 10 to the five LOEL values gave $r^2 = 0.9250$ and 0.8946 for all compounds and non-OPs respectively.

lower value is incorporated into the decision tree as a TTC of 18 μg per person per day (0.3 μg/kg bw/day) for OP structural alerts. Non-OP neurotoxicity would be adequately allowed for by the class III threshold. The introduction of this step is not intended to replace the regulatory framework in place for the approval of OP pesticides. Rather, it is to provide an adjunct for use in cases where a novel, either naturally occurring or anthropogenic, phosphate ester is identified and for which no toxicity data are available. In the case of OP esters it is prudent to apply the TTC principle based on the exposure to all OP esters present in the diet as they are presumed to act via the same mechanism of action. Application of the decision tree would need to take into account the fact that the greatest exposures are likely to be from approved pesticides, which generally have very variable residue levels.

2.2. Consideration of teratogens as a separate class

An additional safety factor (of up to 10-fold) is used for teratogenicity, in certain regulatory approaches, and it might be argued that teratogens should be considered as a separate class when applying the TTC principle. Using data from studies on known teratogens, the NOEL of teratogenicity (T) has been compared to the most sensitive NOEL for embryotoxicity (E) endpoints. A ratio of E/T higher than 1 would reflect the extent to which teratogenicity occurred at lower doses than embryotoxicity. In most cases (Table 2) the E/T ratio was $\leqslant 1$. For seven compounds the ratio was >1; these were all substances with structural alerts for high potency carcinogenicity (e.g. polyhalogenateddibenzo-*p*-dioxins and-dibenzofurans, ethylenethiourea, bromochloroacetonitrile) and would be considered very early in the decision tree (Fig. 2; Steps 1 and 3), before any consideration might be given to a nongenotoxic teratogen. All developmental endpoints, including teratogenicity and embryotoxicity, are considered to be thresholded phenomena. The Expert Group further noted that possible teratogenicity is not considered separately by either the JECFA or the SCF, and that an extra uncertainty factor is not usually applied by these bodies to NOELs for teratogenicity. In addition, an additional safety (uncertainty) factor is not normally applied to teratogenicity by the JECFA and the SCF in setting an ADI, or when they have applied the TTC principle and the Cramer structural class I, II and III thresholds in the safety evaluation of flavours. The Expert Group therefore decided that the application of an additional safety factor for teratogenicity is not needed in the application of the TTC approach.

The NOELs for teratogenicity (Table 2) were compared with 5th percentile NOELs of 3.0, 0.91 and 0.15 mg/kg/day derived by Munro et al. (1996) for Cramer et al. (1978) structural classes I, II and III. Excluding those compounds with structural alerts for high potency carcinogenicity (which would be evaluated for this property in Fig. 2; Steps 2 and 3), the NOELs for teratogenicity in most cases were considerably greater than 3 mg/kg (see Table 2). Therefore, the TTCs for classes I, II and III calculated by dividing the 5th percentile NOELs by the usual 100-fold uncertainty factor would be lower than any threshold related to teratogenicity. Based on these observations the Expert Group decided that consideration of a separate class of teratogens would not be necessary.

3. Consideration of endocrine disrupting chemicals

An important issue in the assessment of the health risks of endocrine disrupting chemicals concerns the reported low-dose effects and dose-response relationships in mammalian species. Low-dose effects refer to biological changes that occur at doses that are lower than those that have been studied in standard tests to evaluate reproductive and developmental toxicity, and at doses that would be well below the conventional NOEL derived from these studies. If low-dose effects were to be established unequivocally this would affect the safety assessment of any compound showing the potential for endocrine disruption, and would also be of importance for the application of a threshold of toxicological concern. The issue of low-dose effects was discussed by the Low-Dose Peer Review Panel and their report was published recently (NTP, 2001). The main overall conclusions of the report were:

- Low-dose effects were demonstrated in laboratory animals exposed to certain endocrine active agents but the effects were dependent on the compound studied and the endpoint measured. In some cases where low-dose effects have been reported, the findings have not been replicated. The validity and toxicological significance of many of these latter observations has not been determined.
- The Low-Dose Peer Review Panel recommended additional research to replicate previously reported key low-dose findings, to characterise target tissue dosimetry during critical periods of development, to identify sensitive molecular markers that would be useful in understanding mechanistic events associated with low-dose effects, and to determine the long-term health consequences of low-dose effects of endocrine active agents.
- The findings of the panel indicate that the current testing paradigm used for assessments of reproductive and developmental toxicity should be revisited to see if changes are needed regarding

dose selection, animal model selection, age when animals are evaluated, and the endpoints being measured following exposure to endocrine active agents.

The SCF in its recent evaluation of bisphenol A (SCF, 2002) concluded that the data on endocrine disrupting effects at very low doses were inconsistent and had not been replicated by subsequent studies. The effects reported

Table 2
Analysis of data on known teratogenic compounds

Compound	Species	Route	Teratogenicity NOEL (mg/kg)	Embryotoxicity NOEL (mg/kg)	Cramer class[a]	Ratio E/T[b]	Reference
Acetazolamide	Rabbit	Oral	50	50	III	1	Nakatsuka et al. (1992)
Acetonitrile	Hamster	Inhal	300	300	III	1	Willhite (1983)
Acetonitrile	Hamster	Oral	200	<100	III	<1	Willhite (1983)
Aflatoxin B1	Hamster	Ip	2	2	III	1	Elis and Di-Paolo (1967)
Antiallergic Sm 857 SE	Rat	Oral	90	90	III	1	Nishimura et al. (1988)
Benomyl	Rat	Oral	31	31	III	1	Kavlock et al. (1982)
Benomyl	Mice	Oral	50	<50	III	<1	Kavlock et al. (1982)
Boric acid	Mice	Oral	248	<248	III	<1	Heindel et al. (1992)
Boric acid	Rat	Oral	55	55	III	1	Price et al. (1996a)
Boric acid	Rabbit	Oral	125	125	III	1	Price et al. (1996b)
Bromo-chloro-acetonitrile (BCAN) (+ tricaprilin as carrier)	Rat	Oral	<5	5	III	>1	Christ et al. (1995)
Butyl benzyl phthalate (BBP)	Rat	Oral	500	500	II	1	Ema et al. (1992)
Chloroform	Rat	Inhal	27	<27	III	<1	Schwetz et al. (1974)
Dichloro-acetic acid (DCA)	Rat	Oral	140	140	III	1	Smith et al. (1992)
Dichloro-acetonitrile (DCAN)) (+ tricaprilin as carrier)	Rat	Oral	15	15	III	1	Smith et al. (1989)
Di (2-ethylhexyl) phthalate (DEHP)	Mice	Oral	190	70	II	0.37	Shiota and Nishimura (1982)
N,N-dimethylformamide	Rat	Oral	166	166	III	1	Hellwig et al. (1991)
Ethylene oxide	Mice	Iv	75	75	III	1	LaBorde and Kimmel (1980)
Ethylene-thiourea (ETU)	Rat	Oral	5	40	III	8	Khera (1973)
Ethylene-thiourea (ETU)	Hamster	Oral	<90	270	III	>3	Teramoto et al. (1978)
Etretinate	Rat	Oral	6	6	II	1	Agnish et al. (1990)
2,2′,4,4′,5,5′ Hexabromo-biphenyl (HBB)	Mice	Oral	21	21	III	1	Welsch and Morgan (1985)
Lithium carbonate	Rat	Oral	50	50	III	1	Marathe and Thomas (1986)
Lithium carbonate	Mice	Oral	200	200	III	1	Szabo (1970)
Mangafo-dipirrisodium (MnDPDP)	Rat	Iv	10μmol	<10μmol	III	<1	Grant et al. (1997)
Manganese chloride	Rat	Iv	5μmol	<5μmol	III	<1	Treinen et al. (1995)
Methanol	Mice	Inhal	986	493	I	0.5	Rogers et al. (1993)
2-Methoxy-ethanol	Rat	Iv	100	<100	I	<1	Sleet et al. (1996)
2-Methoxy-ethanol	Rat	Oral	16	<16	I	<1	Nelson et al. (1989)
2-Methoxy-ethanol	Rabbit	Inhal	4	4	I	1	Hanley et al. (1984)
2-Methoxy-propan-1-ol	Rabbit	Inhal	72	<72	I	<1	Hellwig et al. (1994)
2-Methoxy-propyl-1-acetate	Rat	Inhal	478	478	I	1	Merkle et al. (1987)
2-Methoxy-propyl-1-acetate	Rabbit	Inhal	105	105	I	1	Merkle et al. (1987)
Mirex	Rat	Oral	3	1.5	III	0.5	Khera et al. (1976)
Ochratoxin A	Rat	Oral	0.25	<0.25	III	<0.5	Brown et al. (1976)
1,2,3,7,8-Pentabromo-dibenzofuran (1PeBDF)	Mice	Oral	2	>4	III	>2	Birnbaum et al. (1991)
2,3,4,7,8-Pentabromo-dibenzofuran (4PeBDF)	Mice	Oral	0.8	>4	III	>5	Birnbaum et al. (1991)
Poly-brominated biphenyls	Rat	Oral	200	200	III	1	Beaudoin (1977)
Sodium arsenite	Hamster	Iv	2	2	III	1	Willhite (1981)
Sodium salicylate	Rat	Oral	90	30	I	0.33	Fritz and Giese (1990)
Sodium selenite	Hamster	Oral	14	16	III	1.1	Ferm et al. (1990)
2,3,7,8-Tetrabromo-dibenzo-p-dioxin (TBDD)	Mice	Oral	0.006	>0.19	III	>32	Birnbaum et al. (1991)
2,3,7,8-Tetrabromo-dibenzofuran (TBDF)	Mice	Oral	0.05	0.25	III	5	Birnbaum et al. (1991)
2,3,7,8-Tetrachloro-dibenzo-p-dioxin (TCDD)	Mice	Oral	0.1μg	>3 μg	III	>30	Smith et al. (1976)
Trichlorfon (dipterex)	Mice	Oral	200	<200	III	<1	Courtney et al. (1986)
Trichlorfon (dipterex)	Rat	Oral	375	145	III	0.39	Staples et al. (1976)
Trichloro-acetonitrile (TCAN)	Rat	Oral	35	35	III	1	Christ et al. (1996)
Xylene mixture	Rat	Inhal	2	2	I	1	Mirkova et al. (1983)
Xylene mixture	Mice	Oral	1	1	I	1	Marks et al. (1982)

Inhal—inhalation; Ip—intraperitoneal injection; Iv—intravenous injection.

[a] The Cramer et al. (1978) decision tree is designed primarily for low molecular weight organic compounds.

[b] Ratio of NOEL for embryotoxicity/NOEL for teratogencity. A ratio >1 indicates that teratogenicity occurs in the absence of other forms of embryotoxicity; a ratio <1 arises when the lowest dose tested is a NOEL for teratogencity (T) but a LOEL for embryotoxicity (E); a ratio >1 arises when the lowest dose tested is a LOEL for teratogenicity but a NOEL for embryotoxicity.

at very low doses were not used as the basis for establishing a temporary tolerable intake for this compound. In view of the uncertainties, it seems premature to consider low-dose effects for endocrine disrupting chemicals in the application of a threshold of toxicological concern.

4. Food allergies, hypersensitivity reactions and intolerances

The foods most commonly involved in allergies include cow's milk, egg, fish, crustaceans, peanuts and sesame seeds, and it is believed that there are threshold doses for allergenic foods (Taylor et al., 2002). The most potent allergens are proteins foreign to the host that are capable of inducing immunologically mediated allergic reactions. However, proteins will not be evaluated using the TTC approach. Low molecular weight chemicals may also be involved in allergic reactions by covalently binding to proteins forming a hapten-carrier complex, and thereby becoming a structure potentially able to evoke an immune response. Besides allergic responses to foreign proteins in the food, also non-immunologic effects may occur. These hypersensitivity reactions (pseudo-allergic reactions) and intolerances include direct toxic effects, irritant effects through the epithelium of the gut, or stimulation of mast cell mediator release in a non-immunologic fashion. With this array of mechanisms, and associated symptoms, that are partly overlapping, it is very difficult to develop assays that would predict such adverse reactions to components of the food. Therefore, approaches to predict and quantify such effects are essentially lacking.

For immunologically mediated allergic responses, two levels of quantitative aspects are important: first with respect to the phase of sensitisation, and second with respect to the phase of elicitation of responses in already sensitised individuals, the latter usually requiring much lower amounts of the allergen. A very wide variation of the dose needed to elicit an allergic reaction to a specific allergen exists between individuals sensitised to the same allergen. Although some models of oral sensitisation, notably in the guinea pig and in the BN-rat, have been described, using specific products such as peanut allergen, few dose–response studies have been conducted. There are insufficient dose–response data regarding allergenicity of proteins and low molecular weight chemicals, on which a TTC (or any other assessment) can be based. None of the testing strategies currently available is able to detect allergic responses to low molecular weight chemicals that act as haptens following oral exposure.

5. Metabolic and other toxicokinetic considerations

The toxicity produced by a compound is a function of the chemical groups present, such as nitro-, hydroxyl-, chloro- etc. These groups, and their potential for metabolism, are a central part of the Cramer decision tree (Cramer et al., 1978), which gives rise to the three structural classes used in the decision tree used by the JECFA for the safety evaluation of flavouring substances (JECFA, 1995; Munro et al., 1996). The decision tree comprises a sequence of questions such that compounds with structures indicative of a high potential for toxicity are assigned to structural class III:

- aliphatic secondary amino-, cyano-, N-nitroso-, diazo-, triazeno-, quaternary N;
- unionised substituents containing elements other than C, H, O, N or S (divalent) e.g. halogeno-compounds;
- safrole-like compounds;
- fused lactone or α,β-unsaturated lactone;
- three-membered heterocyclics e.g. epoxides;
- unsubstituted heteroaromatic compounds;
- three or more different functional groups (excluding methoxy- and considering acids and esters as one group);
- unsubstituted aromatic hydrocarbons; and
- compounds without a strongly anionic group for every 20 (or fewer) carbon atoms (for compounds not classified at earlier steps)

Many of these structures are in class III because they undergo metabolic bioactivation to potentially toxic chemical entities, so that metabolic bioactivation is an inherent part of the Cramer classification. In addition some are reactive and/or possess toxic groups that would represent structural alerts for the other TTCs, such as high potency carcinogenicity (Fig. 2; Step 3) and organophosphate (Fig. 2; Step 6), which would be considered at an earlier stage in the TTC decision tree than the TTC for Cramer class III (Fig. 2; Step 8).

Accumulation describes the process by which the amount of a compound in the body increases during repeated dosing, so that the body load after repeated dosage is higher than that after a single dose. The amount of a chemical eliminated from the blood in unit time (e.g. per minute) is the product of clearance (the volume of blood cleared per unit time) and concentration (the amount of chemical per unit volume). For first-order reactions, clearance is a constant value that is a characteristic of the chemical. When the rate of input exceeds the output, which is the product of (clearance×concentration), then the concentration of the compound in the body will increase. When the concentration has increased such that (clearance×concentra-concentration) equals the rate of input there will be a constant concentration, which is known as the steady-state condition. The extent of accumulation reflects the relationship between the body-burden after a single dose compared with the steady-state condition.

All chemicals will accumulate if the dosage is more frequent than every 1–2 half-lives. Species differences in clearance will determine the difference in steady-state body burden between experimental animals and humans, and also the time taken to reach the steady-state condition (Renwick, 2000). Animals usually metabolise chemicals more rapidly than humans, and therefore their steady-state body-burdens will be reached more rapidly and also will be lower than those in humans for the same input (on a mg/kg basis). This difference is one of the reasons for the use of an inter-species uncertainty or safety factor when converting the NOEL from a chronic animal study into an ADI or RfD. The thresholds developed by Munro et al. (1996) based on the Cramer classification (Cramer et al., 1978) incorporate the default 100-fold uncertainty factor used for deriving an ADI. Therefore the usual differences in body burden between animals and humans, arising from different rates of metabolism, would have been allowed for. The use of an ultra-conservative linearised low-dose extrapolation for carcinogens to the lifetime risk level of 10^{-6}, from which the TTC values in Fig. 2; Steps 4 and 5 are derived, is considered to allow for any species differences in metabolism and toxicokinetics. Most genotoxic chemicals, and certainly those that would be considered using a TTC approach, would require metabolic bioactivation to act as carcinogens, and the species differences in the rates of metabolism could result in greater activation in animals than in humans.

The presence of a C-, N-, O-, S- or P-containing functional group within a molecule provides a polar centre and also a potential site for metabolism. In consequence most chemicals with potentially toxic groups are metabolised rapidly, and in vivo accumulation is not a concern.

Molecules that do not possess functional groups, for example simple linear alkanes and polycyclic aromatic hydrocarbons, will be eliminated readily, either by exhalation (low molecular weight volatile compounds) or by metabolism (long-chain aliphatic and polycyclic aromatic compounds). In consequence in vivo accumulation is not a concern; the potential toxicity will have been taken into account in the Cramer decision tree, in the use of structural alerts for genotoxicity and in the use of the 100-fold factor in the Munro et al. (1996) approach.

Halogen substituted carbon atoms (structural class III) are not readily metabolised, and a halogen substituent may block metabolism at that carbon atom or at adjacent carbon atoms. The absence of metabolism, combined with the increased lipid solubility of the molecule, can give rise to accumulation. Groups that may be associated with reduced rates of metabolism and increased accumulation are

1. halogeno-substituents on alkyl groups—such that all hydrogen atoms are replaced, for example a-CF_3 group; and
2. halogeno-substituents on aromatic rings—such that all ring-carbon atoms either have a halogen substituent, or are adjacent to a halogen-substituted ring-carbon atom

Polyhalogenated compounds without other functional groups present in the Munro et al. (1996) database include chlordane, heptachlor, hexabromobenzene, hexachlorobenzene, hexachlorobutadiene, hexachlorethane, hexachlorophene, mirex and a number of halogeno-derivatives of methane, ethane and ethylene. Of these compounds only chlordane and hexachlorobenzene had NOELs below the 5th percentile used to calculate the TTC for class III compounds (the differences were only 3-fold and 2-fold, respectively). Molecules with a high proportion of halogen substituents, but which still retain some sites for hydroxylation or the formation of an epoxide, would show slow metabolism, but oxidation could still occur at available, non-substituted carbon atoms. Such molecules would be retained to a greater extent than non-halogenated analogues, but would not show the very long half-lives of fully substituted compounds. The rate of metabolism at the available carbon atoms may show species differences, but this will have been taken into account in the use of the 100-fold factor.

Accumulation needs special consideration when there are major species differences in clearance, and hence in steady-state body loads, which exceed the usual uncertainty factor for species differences. Differences in the extent of accumulation of polyhalogenated aromatic compounds, such as TCDD, in rats and humans would not be allowed for by the usual 10-fold interspecies uncertainty factor. Recent evaluations of dioxins by the WHO, EPA and SCF have been based on the estimated body burden at steady state rather than the daily intake, in order to allow for species differences in bioaccumulation. In consequence, the TTC for a non-genotoxic class III compound would not be appropriate for compounds such as TCDD for which the extent of accumulation shows very large species differences. In addition, TCDD and related structures, such as polyhalogenated-dibenzofurans and-biphenyls, were not included in the compounds analysed by Munro et al. (1996) to derive the TTC for class III compounds. In relation to chemical risk, accumulation would also be of concern when the half-life was so long that the steady-state body load was not reached in subchronic studies in animals and a chronic study had not been performed in animals, but human exposure was throughout life. Many heavy metals are known to accumulate, e.g. cadmium accumulates in the kidney. There are extensive databases on most heavy metals and these should be used for risk assessment. For example a new food source of lead would not be considered using the TTC decision tree, and the possible health impact of any additional intake

would be considered using usual risk assessment procedures. The TTC could be a useful approach, in the absence of chemical-specific toxicity data on a previously unstudied metal, for example if a safety decision was required urgently following the discovery of trace levels of a lanthanide in food. However, it should be appreciated that metals were not in the database used by Munro et al. (1996) to define the TTCs used in the current decision tree. In consequence, metals in elemental, ionic and organic forms would not normally be evaluated using the decision tree.

Major species differences in clearance, which exceed the usual inter-species safety/uncertainty factor, would be of concern whether or not the compound accumulated during chronic treatment. Currently it is not possible to predict which halogenated compounds would show species differences in clearance (and hence in steady-state body load) greater than allowed for by the inter-species toxicokinetic factor that was part of the 100-fold factor used by Munro et al. (1996) in the development of the structural class thresholds. However, this criticism would also apply to the application of the usual default uncertainty factor to the NOEL for such a compound with a comprehensive toxicity database.

In conclusion, specific considerations of metabolism and accumulation are not necessary in the application of a TTC, providing that the decision tree is not applied to substances that are likely to show very large species differences in accumulation, such as polyhalogenated dibenzo-*p*-dioxins and related compounds, or metals which have extremely long half-lives and were not included in the Munro et al. (1996) database.

6. Exposure in relation to the TTC

As discussed earlier the threshold for regulation, as used by the US Food and Drug Administration for the low exposures to food contact materials, is a concentration of a compound in food below which no regulation of the compound is considered necessary. It is assumed that the compound is present in the whole diet, e.g. in 1.5 kg of food and 1.5 kg of beverages consumed daily. In contrast, the thresholds used in the TTC approach are intakes, expressed per person/day or per kg/bw per day, below which a given compound is not expected to present a toxicological concern. Therefore, the TTC for a given compound has to be compared with a human exposure estimate for the compound.

As an example, when the compound under consideration has a TTC of 90 μg per person per day (e.g. non-genotoxic, non-OP and belongs to the Cramer structural class III) and the compound occurs uniformly in the whole diet (1.5 kg of food and 1.5 kg of beverages), this intake is reached by concentrations in the diet of 30 μg/kg. However in cases where a given compound will not be present in the whole diet but only in a specific product, the human total exposure to this compound is determined by its concentration in the product and the amount of the product that is actually consumed daily by users of the product. When the compound is present only in beverages (e.g. 1.5 kg fluids) but does not occur in food, the above-mentioned TTC is equivalent to a concentration of 60 μg/kg drinking water. When the only route of exposure is via ingestion of a single food product, which is consumed in daily amounts of 100 g, the TTC of 90 μg per person per day would be reached by concentrations of 900 μg/kg of the compound in that food.

In certain cases it will be necessary to consider combining the exposures to substances, which are thought to possess a common mechanism of action, such as OP esters. Simple addition of the intakes will not allow for differences in potency or interactions, and will assume that the risk from each compound, based on its structural characteristics, is not altered by the presence of the other compounds. In addition relevant exposures from sources other than food should be taken into account. When dealing with complex mixtures of diverse chemicals, assessment using the TTC approach should focus on the exposure to a "marker" compound or major compound (which represents a high proportion of the mixture and is in the highest Cramer class of the known constituents of the mixture).

In conclusion, for applying the TTC principle, an appropriate total human exposure estimate is necessary. As the TTCs are expressed in terms of μg per person per day, special consideration may need to be given to products designed for specific groups such as children, and both the intake estimates and the TTC may need to be related to body weight.

7. Explanation and use of the TTC decision tree

Application of the TTC principle should be seen as a preliminary step in safety assessment. The decision tree and the TTC principle are designed as structured aids to expert judgement and should be applied only by those who have a sufficient understanding of toxicology principles and chemical risk assessment. The output from the decision tree is either that the anticipated exposure would not be predicted to represent a safety concern, or that risk assessment is not appropriate without toxicity data on the compound. In the latter circumstances the results of the decision tree could be used to give advice to risk managers about the extent to which exposure would have to be reduced to give a negligible risk. As mentioned previously, the TTC principle can also be applied to indicate analytical needs or to set priorities in toxicity testing.

Prior to application of the TTC approach, all available toxicity data on the compound should be collected and

evaluated (Renwick et al., 2003). The TTC approach should be used only in cases where the available chemical-specific data are inadequate for normal risk characterisation. Any available information on the compound should be considered at the same time as the decision tree is applied, to ensure that any decision is compatible with the available data. The TTC is not designed to replace conventional approaches to risk characterisation for established and well-studied chemicals, such as food additives and pesticides. In addition, because of the nature of the databases used to derive the different TTC values, the approach would not normally be applied to:

1. heavy metals, such as arsenic, cadmium, lead and mercury, for reasons mentioned before;
2. compounds with extremely long half-lives that show very large species differences in bioaccumulation, such as TCDD and structural analogues; or
3. proteins

The decision tree has been developed in order of decreasing potency, so that compounds for which a TTC is inappropriate are eliminated from the decision tree at an early stage. Following this step, compounds with intakes so low that they would not raise concerns, irrespective of the functional groups present in the molecule, are considered not to represent a safety concern, and are not subject to further detailed consideration. This is followed by a series of questions designed to identify structural characteristics indicative of decreasing potency and with increasing TTC values.

The decision tree (see Fig. 2) considers in the first steps the potential for genotoxicity and removes from further consideration chemicals with the structural alerts found in the most potent genotoxic carcinogens. Later in the decision tree, adverse effects that would show a threshold in the dose response curve are addressed. Historically, hazard characterisation of non-threshold and threshold effects have adopted different approaches to the establishment of intakes that would be without significant adverse health effects. These different approaches have been maintained in the selection of the different TTC values given at different parts of the decision tree.

For the risk characterisation of genotoxic compounds the carcinogenicity dose–response data in experimental animals have been extrapolated from the experimental range down to an incidence that would be considered to be a negligible or de minimis risk, such as a 1 in 10^6 risk in a lifetime. The use of low-dose extrapolation is not accepted as valid by all regulatory authorities, because the numerical value of the dose associated with a 1 in 10^6 risk is determined largely by the mathematical model applied to the dose–response data. However, while the numerical value may be model-dependent, it is generally accepted that the use of a linearised low-dose extrapolation, as used to derive the TTC values in the decision tree (Fig. 2; Steps 4 and 5), would represent a "worst-case" analysis, and that the true incidence at the calculated dose would be somewhere between zero and 1 in 10^6 (Barlow et al., 2001; Edler et al., 2002).

The database from which the thresholds were calculated includes compounds that were selected for a chronic cancer bioassay largely because of structural class and/or high production volume. In consequence there was a high probability that selected compounds would be carcinogens (Cheeseman et al., 1999), compared with an estimate of 5–10% for general chemicals in production (Fung et al., 1995). The thresholds that have been proposed at Steps 4 and 5 are based on a highly conservative methodology, which incorporates a number of conservative aspects (Box 1).

After excluding proteins, heavy metals and polyhalogenated-dibenzodioxins and related compounds in the first question of the decision tree, the second question in the decision tree (Fig. 2; Step 2) separates compounds with structural alerts for potential genotoxicity from non-genotoxic compounds. Compounds with structural alerts for genotoxicity, which also contain functional groups associated with high carcinogenic potency (the genotoxic COC) are removed from the decision tree. Different TTC values related to carcinogenic risk are used for potential genotoxic compounds that are not in the genotoxic COC (Fig. 2; Step 4), and for compounds that do not have structural alerts for genotoxicity (Fig. 2; Step 5).

Box 1. Conservative assumptions in the use of the Carcinogenic Potency Data Base to derive TTC values that would allow for any carcinogenic risk.

- Establishment of the dose giving a 50% tumour incidence (TD50) using data for the most sensitive species and most sensitive site (Cheeseman et al., 1999).
- Based on a selected subset of the database containing 730 carcinogenic substances which had adequate estimates of the TD50 following oral dosage.
- Simple linear extrapolation from the TD50 to a 1 in 10^6 incidence.
- The approach assumes that all biological processes involved in the generation of tumours at high dosages are linear over a 500,000-fold range of extrapolation.
- Simple linear low-dose extrapolation is conservative because the possible effects of cytoprotective, DNA repair, apoptotic and cell cycle control processes on the shape of the dose-response relationship are not taken into account.
- All of the compounds were analysed assuming there is no threshold in the dose–response.

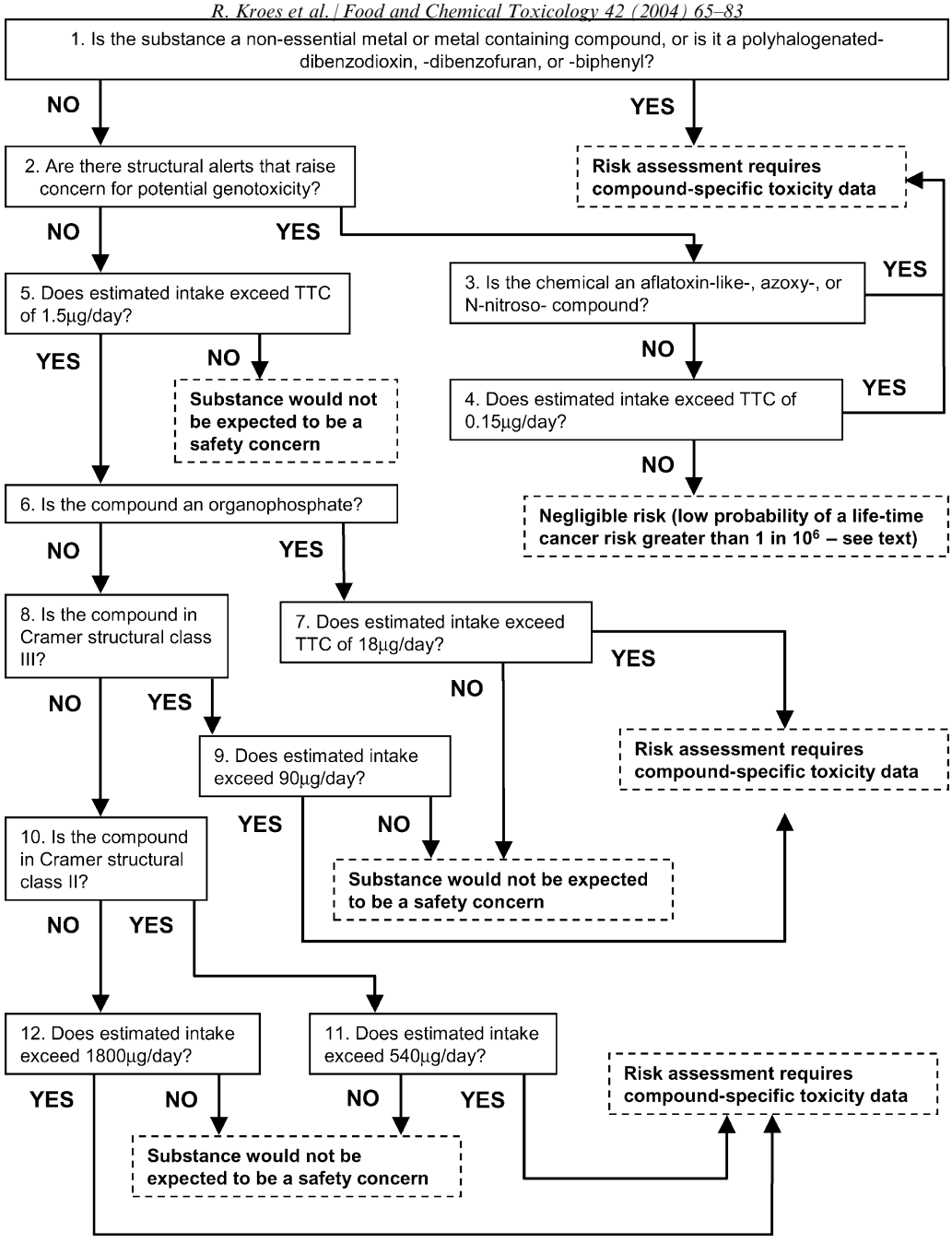

Fig. 2. Decision tree for low molecular weight compounds for which limited toxicity data are available, that incorporates different thresholds of toxicological concern related to different structural characteristics.

For non-genotoxic compounds the approach normally adopted is to establish the NOEL from an evaluation of the database on the compound and to use this as the starting point for risk characterisation. For such compounds, it is considered that toxicity will occur when the dosage and effects are sufficient to exceed normal homeostatic processes. A consequence of this is that it is possible to establish a maximum intake that would not alter normal homeostasis and would not produce an effect. The usual approach to calculate the safe intake for humans is to divide a NOEL from a study in animals by an uncertainty factor of 100.

The thresholds at Steps 9, 11 and 12 in the decision tree are based on analysis of the 5th percentile of the distributions of NOELs for compounds in structural class I (137 compounds), class II (28 compounds) and class III (448 compounds) in the database of Munro et al. (1996), with the NOELs from chronic animal studies divided by a 100-fold factor, and the NOELs from sub-chronic studies divided by a 300-fold factor. The calculated thresholds for structural classes I, II, and III are 1800 μg/day (30 μg/kg bw/day), 540 μg/day (9 μg/kg bw/day) and 90 μg/day (1.5 μg/kg bw/day) respectively. If the intake of a compound is estimated to

be below the relevant structural class I, II or III threshold, there is a 95% probability that the intake would be below the compound-specific health-based guidance value that would be determined if data from a chronic animal bioassay were available on the compound.

The structural class for a compound is determined using the Cramer et al. (1978) decision tree. Although this decision tree has been used primarily for the classification of flavouring substances, the TTC values derived by Munro et al. (1996) were based on an analysis of 613 compounds representing a diverse array of chemical structures. Therefore, the analysis by Munro et al. (1996) supports the classification system of the Cramer et al. (1978) decision tree for a diverse array of chemicals structures. Concerns about the reliability of the Cramer et al. classification would arise if highly potent structures were to be allocated to structural class I or II. Analysis of the database of Munro et al. (1996) for "misclassification" showed that only three compounds in structural class I had calculated NOELs less than the 5th percentile NOEL for class II, and only one was below the 5th percentile NOEL for class III. None of the class II compounds had a NOEL below the 5th percentile NOEL for class III.

8. Decision tree

The decision tree (Fig. 2) is based on toxicity data for compounds of low molecular mass and with known chemical structure. All outputs of the decision tree are based on the estimated intake or exposure that was used to determine whether or not a TTC is exceeded. Re-evaluation would be necessary if additional intake data or estimates differed from those used in the initial evaluation.

The decision tree starts with a consideration of genotoxicity and carcinogenicity in relation to structural alerts and intake.

Step 1. This Step removes from consideration types of compound and chemical structures that are not adequately represented in the carcinogenicity and toxicity databases that are the basis for the TTC values in subsequent Steps.

Step 2. If the answer to Step 1 is NO, then Step 2 identifies compounds that have the potential for genotoxicity and could be possible genotoxic carcinogens.

Step 3. If the answer at Step 2 is YES (there are structural alerts for genotoxicity), then Step 3 identifies those structures that are likely to be the most potent genotoxic carcinogens. Analysis of the dose–response data for compounds in Table 1 identified aflatoxin-like-, azoxy-, N-nitroso- compounds, 2,3,7,8-dibenzo-*p*-dioxin and its analogues and steroids as being the most potent compounds (the COC—see above). Steroids were excluded at this stage because the subsequent Step (Step 4) involves a TTC derived using linear low-dose extrapolation, which is not an appropriate risk assessment approach for compounds that would show thresholds in their dose-response relationships. Analogues of 2,3,7,8-dibenzodioxin will have been excluded from consideration at Step 1. Aflatoxin-like-, azoxy- and N-nitroso-compounds, which were identified as the COC for high potency genotoxic carcinogens, would be excluded from further consideration at this Step because there would be a high probability of a significant carcinogenic risk at intakes below the threshold in Step 4.

Step 4. All compounds evaluated at this Step would be considered to be potential genotoxic carcinogens, but with the most potent structures removed at Steps 2 and 3. The TTC of 0.15 μg/day (0.0025 μg/kg bw/day) is based on the analysis of the dose–response data for carcinogenic compounds summarised in Table 1. For the different structural groups in Table 1 that would be evaluated at Step 4, this threshold gives a 86–97% probability that any risk would be less than 1 in 10^6 if the intake were at or below the TTC, and the compound were to be a genotoxic carcinogen. Because of the conservatism and assumptions in the linearised low-dose extrapolation used to derive this value (see Box 1), the probability that any risk would be less than 1 in 10^6 is likely to be considerable higher than is indicated by the data in Table 1. Only 15 of 730 (approx. 2%) of the compounds in the carcinogenic potency database assessed by this decision tree would reach Step 4 and give a theoretical risk greater than 1 in 10^6 at an intake equal to a TTC of 0.15 μg/day (0.0025 μg/kg bw/day). The inclusion of this Step is NOT designed to allow genotoxic compounds to be added deliberately to the food supply, but rather to determine if there is a significant safety concern if they are detected in food, for example as a contaminant. Because of the selection at earlier Steps and the conservative approach adopted, there would be negligible risk at intakes less than the TTC in Step 4.

Step 5. If the answer at Step 2 is NO (there are no structural alerts for genotoxicity), this Step means that compounds with very low exposures would not require structural classification and further detailed consideration. The TTC of 1.5 μg/day (0.025 μg/kg bw/day) (Flamm et al., 1987; Rulis, 1989; Federal Register, 1993) is based on an analysis of carcinogenicity databases, which included both genotoxic and non-genotoxic compounds, and assuming a linear low-dose extrapolation from the TD_{50} to a 1 in 10^6 risk. Based on the analysis by Munro (1990) this threshold gives a 96% probability that any risk would

be less than 1 in 10^6 if the intake were at or below the TTC and 10% of the compounds reaching this Step were genotoxic carcinogens. The use of the TTC of 1.5 μg/day is extremely conservative in the present context because all compounds with known structural alerts for genotoxicity will have been evaluated using Steps 3 and 4. The use of linearised low-dose extrapolation would be highly conservative and largely inappropriate for any non-genotoxic carcinogenic compound reaching this Step. Because of the selection at earlier Steps and the conservative approach adopted, there would not be a safety concern if the intakes of compounds, including any unrecognised carcinogenic compounds, evaluated at this Step were less than the TTC of 1.5μg/day (0.025 μg/kg bw/day). The TTCs for other forms of toxicity considered in subsequent Steps are greater than 1.5 μg/day and therefore other forms of toxicity would not be a concern at intakes at or below this TTC.

Step 6. This Step identifies the major functional group, organophosphates (OPs), for which the dose-response data indicate greater potency than for Cramer class III, and which require consideration before class III. The inclusion of this Step is not intended to replace the normal regulations and controls on pesticide OPs, or for them to be added deliberately for some technical reason to the food supply, but rather to determine if there is a significant safety concern were a non-approved or unregulated OP to be detected in food, for example as a contaminant. Although unlikely, novel biologically-derived phosphate esters of organic compounds may be detected in food in the future because of improvements in analytical sensitivity, and this TTC would provide a basis for an initial safety evaluation.

Step 7. The TTC for OPs (18 μg per day or 0.3 μg/kg bw/day) was established from a cumulative plot of the NOELs for OPs, and the establishment of the approximate 5th percentile of the distribution. This 5th percentile NOEL was divided by the usual uncertainty (safety) factor of 100-fold to determine a TTC expressed as μg/kg/day and then converted to a 60 kg body weight. This TTC is based on a less comprehensive database than those for structural classes I, II and III. Because of the selection at earlier Steps and the conservative approach adopted there would not be a safety concern if the intakes of an OP contaminant that reached this Step were less than the relevant TTC.

Step 8. This Step ascertains if the compound, which is not an organophosphate, contains functional groups that have not been considered previously, but which may be indicative of significant toxicity (Cramer structural class III).

Step 9. If the answer at step 8 is YES (the compound is in class III) this question determines if the intake exceeds the TTC for class III of 90 μg/day (1.5 μg/kg bw/day). Because of the selection at earlier steps and the conservative approach adopted, there would not be a safety concern if the intakes of a class III compound were at or below an intake of 90 μg/day (1.5 μg/kg bw/day).

Step 10. This Step ascertains if the compound contains functional groups that have not been considered previously, but which may be indicative of some toxicity (Cramer structural class II).

Step 11. If the answer at Step 10 is YES (the compound is in class II) this question determines if the intake exceeds the TTC for class II of 540 μg/day (9 μg/kg bw/day). Because of the selection at earlier Steps and the conservative approach adopted, there would not be a safety concern if the intakes of a class II compound were at or below an intake of 540 μg/day (9 μg/kg bw/day).

Step 12. If the answer at Step 10 is NO (the compound is not in class II) this question determines if the intake exceeds the TTC for class I of 1800 μg/day (30 μg/kg bw/day). Because of the selection at earlier Steps and the conservative approach adopted, there would not be a safety concern if the intakes of a class I compound were at or below an intake of 1800 μg/day (30 μg/kg bw/day).

9. Conclusions

The application of the TTC principle is recommended for substances present in food at low concentrations which lack toxicity data, but for which exposure analysis can provide sound intake estimates. The use of a decision tree (Fig. 2) provides a structured approach that allows consistent application of the TTC principle to the risk of chemicals in food. In addition, the present paper considers a number of further questions related to the application of the TTC principle as a follow up of an earlier published paper (Kroes et al., 2000). Consideration is given to an increased safety assurance by the identification of structural alerts for high potency carcinogens and by answering the issues of whether neurotoxins, teratogens or endocrine disrupting chemicals should be considered as separate classes. In addition, it was investigated whether food allergies, hypersensitivity reactions and intolerances fit into the TTC concept. Finally the Expert Group evaluated if a separate consideration of metabolism and accumulation was necessary in the application of a TTC. Since the databases that were used to derive the TTC values did not include toxicity data on proteins or heavy metals such as cadmium, lead and mercury the decision tree should not be used for such substances. In addition polyhalogenated dibenzo-*p*-dioxins,-dibenzofurans and

-biphenyls are excluded from consideration because the linearised low-dose method used for estimation of cancer risk (Step 4) is not appropriate, and because the TTC values for threshold (non-genotoxic) effects would not allow for very large species differences in elimination, and because similar compounds were not in the database of Munro et al. (1996), which was used to derive the TTC values in Steps 9, 11 and 12.

The TTC principle represents an important pragmatic tool for risk assessors, risk managers and industry to allow the prioritisation of resources to compounds with high exposures and/or high toxicity. It can accelerate the evaluation process of substances to which humans are exposed at low levels. The application of the TTC principle will allow resources used in food safety assessment to be focused on those chemicals of greatest public health importance and will reduce the number of animal toxicity studies. The decision tree described in the paper should become an important part of any chemical prioritisation procedure, or preliminary risk assessment, which is based on minimal chemical-specific toxicity data and which depends on the use of data on structural analogues.

The TTC principle is applicable to other sectors of health risk assessment such as in occupational and environmental settings and may also be further developed for environmental risk assessment. For example, the recent Report of the Royal Commission on Environmental Pollution in the UK (Royal Commission, 2003) highlighted concerns that humans are exposed to a diverse array of chemicals, and that there is a urgent need for the evaluation of a large number of chemicals, while at the same time reducing the reliance on animal experimentation. An increasing reliance on in vitro and in silico methods for hazard identification was proposed by the Royal Commission. However, the classification of chemicals as "of concern" based solely on hazard identification without taking into account potential intake and considerations of predicted in vivo potency, could lead to an unnecessarily large number of compounds requiring extensive hazard characterisation using in vivo animal experimentation. The TTC provides a method by which assessment of the potential risk to human health can be based on any available data (including in vitro or in silico information) combined with information on potential intake and the predicted in vivo toxicity, based on data for compounds that share similar chemical structures. The TTC principle could also be used to indicate analytical data needs and to set priorities for levels of "inherent concern". In addition, because the safety conclusions reached using the decision tree relate to daily intake throughout life, the approach could be used in the assessment of impurities present of compounds such as pesticides and drugs or their formulations.

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Prof. A. Carere	Institute of Public Health	I
Dr. M. Cheeseman	Food and Drug Administration	USA
Prof. E. Dybing	Norwegian Institute of Public Health	N
Prof. C.L. Galli	University of Milan	I
Prof. Dr. W. Grunow	Federal Institute for Risk Assessment (BfR)	D
Dr. T. Kimura	Ajinomoto	JPN
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Prof. J-C Lhuguenot	University of Bourgogne—ENSBANA	F
Dr. I. Mangelsdorf	Fraunhofer Institute of Toxicology and Aerosol Research	D
Prof. P. Mantle	Imperial College of Science, Technology, and Medicine	UK
Dr. I. Munro	CanTox Health Sciences International	CDN
Dr. J. O'Brien	Groupe Danone	F
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Prof. P. Tobback	Catholic University of Leuven	B
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