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The Threshold of Toxicological Concern (TTC) concept: Development and regulatory applications

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Content

List of abbreviations

ADI	Acceptable Daily Intake
ALARP	As Low As Reasonably Practicable
BW	Body Weight
CHMP	Committee for Medicinal Products
COLIPA Association	European Cosmetic, Toiletry and Perfume
CPDB	Carcinogenic Potency Database
CVMP	Committee for Medicinal Products for Veterinary Use
DNEL	Derived No Effect Level
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EFSA	European Food Safety Authority
EMA	European Medicines Agency
FVST	Danish Veterinary and Food Administration
GEV	Generic Exposure Value
GHS	Globally Harmonised System of Classification and Labeling of Chemicals
GLEV	Generic Lowest Exposure Value
HMPC Products/Preparations	Committee on Herbal Medicinal
ILSI	International Life Sciences Institute
JECFA Additives	Joint FAO/WHO Expert Committee on Food
LD	Lethal Dose
LLNA	Local Lymph Node Assay
LO(A)EL/C Level/Concentration	Lowest Observed (Adverse) Effect

MOE	Margin of Exposure
MOS	Margin of Safety
MRL	Maximum Residue Level
MSDI	Maximised Survey-Derived Intake
MST	Danish Environmental Protection Agency
NO(A)EL/C	No Observed (Adverse) Effect Level/Concentration
OEL	Occupational Exposure Limit
OP	Organophosphates
QRA	Quantitative Risk Assessment
(Q)SAR	(Quantitative) Structure Activity Relationship
REACH	Registration, Evaluation, and Authorization of CHemicals
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCF	Scientific Committee for Food
SCHER	Scientific Committee on Health and Environmental Risks
TD	Tumourigenic Dose
TDI	Tolerable Daily Intake
TGD	Technical Guidance Document
TR	Threshold of Regulation
TTC	Threshold of Toxicological Concern
US-FDA	US Food and Drug Administration

Preface

The concept, that levels of exposure for chemicals can be defined below which, there are no significant risks to human health, is widely accepted, both for carcinogenic and non-carcinogenic endpoints. From this, the concept of the Threshold of Toxicological Concern (TTC) has arisen, which refers to the establishment of a human exposure threshold value for chemicals, below which there would be no appreciable risk to human health assuming lifetime exposure.

The TTC concept is intended to be used as a substitute for missing substance specific toxicological information in situations where the human exposure is judged to be so low that the undertaking of elaborate toxicity studies is considered inappropriate for reasons of manpower, cost and animal welfare.

This project was initiated in order to evaluate if and how a TTC concept can be applied for regulatory purposes by the Danish Environmental Protection Agency (MST) and the Danish Veterinary and Food Administration (FVST) instead of traditional risk assessments for human health effects of xenobiotics present in food or environment at low levels.
The aim of this report is therefore to give an overview of the TTC approaches available, evaluate the scientific data behind the concepts including the uncertainties, and to address the regulatory applicability within the MST and FVST resort areas.

The report has been prepared by Elsa Nielsen and John Christian Larsen (Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark). The authors are solely responsible for the recommendations and conclusions presented in the report, which do not necessarily reflect the positions of the involved institutions.

The report has been subjected to review and discussion in a steering committee with representatives from the following Danish authorities/ institutions:

The Danish Environmental Protection Agency
The Danish Veterinary and Food Administration
The National Food Institute, Technical University of Denmark

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Summary

The assumption that there is a level of exposure for chemical substances below which there is no adverse toxicological effects, and thus no significant risks to human health, is widely accepted for non-carcinogenic endpoints. For compounds that are both genotoxic and carcinogenic, it is believed that there are levels of exposure below which the risk to human health is minimal. From this, the concept of the Threshold of Toxicological Concern (TTC) has arisen, which refers to the establishment of a human exposure threshold value for chemicals, below which there would be no appreciable risk to human health assuming a lifetime exposure.

The establishment of the TTC concept is based on the analysis of the toxicological data and chemical structures of a broad range of different chemical substances. The concept might be used as a substitute for substance specific toxicological information in situations where there are limited or no information on a given substance to which the human exposure is negligible or so low that undertaking toxicity studies is considered not warranted, because of the costs incurred in the use of animals, manpower and laboratory resources as well as for animal welfare reasons.

Historical development

So far, two principal approaches have been used in the TTC concept developed to date: The general TTC concept and the tiered TTC concepts related to structural information and/or metabolic and toxicological data of individual substances.

The general TTC concept, intended to cover also carcinogenic effects of chemicals, was introduced by Rulis (1986, 1989) of the US Food and Drug Administration (US-FDA) as a 'Threshold of Regulation' (TR, described in section 3.1). Rulis used data on a subset of 343 oral carcinogens from animal studies compiled in the Carcinogenic Potency Database (CPDB) (Gold et al. 1984). From this Rulis initially proposed, for illustration, a threshold value of 0.15 µg/person/day. Subsequently, Munro (1990) confirmed the proposal of Rulis and included more rodent carcinogens in the original database, bringing the total to 492 rodent carcinogens (Gold et al. 1989). The robustness of the database was confirmed by Cheeseman et al. (1999) who expanded the data set to 709 carcinogens based on the continuously updated CPDB database (described in section 3.3). Munro (1990) concluded that a threshold value of 1.5 µg/person/day would provide a high degree of health protection. Subsequently the US-FDA adopted this threshold value and derived a dietary concentration of 0.5 µg/kg food as the TR for substances used in food-contact articles (Federal Register, 1993, 1995).

Munro et al. (1996) explored the relationship between chemical structure and toxicity through the compilation of a reference database consisting of 613 chemical substances tested for a variety of non-carcinogenic toxicological endpoints in rodents and rabbits in oral toxicity tests, including studies on sub-chronic, chronic, reproductive and developmental toxicity (described in

section 3.2). For many of the substances, more than one NOEL was identified and in all, the reference database contained 2941 NOELs. The substances were grouped into one of three potency classes (Class I, II, and III) based on the chemical structure using the decision tree developed by Cramer et al. (1978) (described in section 3.2). Cumulative distributions of the logarithms of NOELs were plotted separately for each of the structural classes (Figure 1 in section 3.2). The 5th percentile NOEL was estimated for each structural class and this was in turn converted to a human exposure threshold by applying the conventional default safety factor of 100.
The structure-based, tiered TTC values established were 1800 µg/person/day for Cramer Class I substances, 540 µg/person/day for Cramer Class II substances, and 90 µg/person/day for Cramer Class III substances (Table 3 in section 3.2). Endpoints covered include systemic toxicity except mutagenicity and carcinogenicity. Later work increased the number of chemicals in the database from 613 to 900 without altering the cumulative distributions of NOELs.

In later analyses Kroes et al. (2000, 2004), combined the general TTC concept and the structure-based, tiered TTC concept to develop an enhanced structure-based, tiered TTC approach (described in section 3.4).
The TTC value of 1.5 µg/person/day (0.025 µg/kg bw/day), used by US-FDA in the Threshold of Regulation (TR) policy, had been further analysed by Cheeseman et al. (1999) who using an extended database concluded that there may be some chemicals with a very high carcinogenic potency that may not be covered (described in section 3.3). Five groups of compounds (Table 4 in section 3.4) were identified having a significant fraction of their members that may still be of concern at an intake of 0.15 µg/person/day (0.0025 µg/kg bw/day), which is 10-fold below the TR value. Three of these groups comprised substances that are genotoxic: the aflatoxin-like, azoxy- and ***N***-nitroso-compounds, while two groups contained substances that were non-genotoxic: 2,3,7,8-dibenzo-***p***-dioxin (TCDD) and its analogues (dioxins), and the steroids.

Neurotoxicants, immunotoxicants and teratogens were also further explored (described in section 3.4), and it was concluded that except for the neurotoxicants being organophosphate pesticides such compounds would be covered by the structure-based, tiered TTC approach. For organophosphates, a human exposure threshold of 18 µg/person/day was derived. This threshold for organophosphates was not intended to replace the normal regulatory assessments and controls for organophosphates used as pesticides, but could be used to evaluate the risk should a non-approved or unregulated organophosphate be detected as a contaminant in food.

The human exposure thresholds (TTC values) established by Kroes et al. (2004) are summarised in the table below this paragraph (identical with Table 5 in section 3.4). As already mentioned, the aflatoxin-like compounds, azoxy-compounds, ***N***-nitroso-compounds, 2,3,7,8-dibenzo-***p***-dioxin (TCDD) and its analogues, and steroids are not included in the TTC concept due to their carcinogenic potencies. 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 TTC concept should not be used for such substances either. Compounds with extremely large half-lives that show very large species differences in bioaccumulation, such as TCDD and analogues were not in the original database of Munro et al. (1996) and are also excluded from consideration by the TTC concept. In addition, although the data

available do not permit the establishment of a clear causal link between endocrine active chemicals and adverse effects in humans, the issue of potential low-dose effects of hormone-like chemicals remains unresolved, and therefore endocrine active chemicals should not be evaluated using the TTC concept.

Human exposure threshold (of toxicological concern) (TTC) values (After Kroes et al. 2004).

Type of chemical	µg/person/day	µg/kg bw/day
Genotoxic compounds	0.15	0.0025
Non-genotoxic compounds	1.5	0.025
Organophosphates	18	0.3
Cramer Class III	90	1.5
Cramer Class II	540	9
Cramer Class I	1800	30

The TTC concept established by Kroes et al. (2004) has recently been amended by Felter et al. (2009) to allow for inclusion of data from Ames test on chemicals with structural alerts for genotoxicity and to include short-term exposures (Table 10 in section 5.2.4).

The TTC concept at present is limited to exposures to chemicals via the oral route as the databases behind the concept do not include studies using dermal application or exposure by inhalation. A promising attempt to extend the TTC concept to cover inhalation exposure has been published by Escher et al. (2008a,b, 2010) based on analyses performed with the RepDose database developed at the Fraunhofer Institute for Toxicology and Experimental Medicine (described in section 5.9.2 and 5.9.4). Application of the TTC concept for dermal exposure has been discussed in the context of cosmetic ingredients (described in section 5.5.1). These attempts to extend the TTC concept to cover inhalation and dermal exposure may, after refinement and validation, in the future be applicable for assessment of substances in cases where these exposure routes are relevant, but are considered too limited for regulatory uses for the time being.

As the databases behind the TTC concept are exclusively based on systemic effects after repeated oral administration of the chemicals, acute toxicity and local effects such as irritation and sensitisation are not covered. The traditional threshold approach has never been applied to allergenicity, nor has a NOEL based on allergy ever been established and thus, also endpoints like allergic reactions, hypersensitivity, and intolerance are not covered. A proposal for use of the TTC concept for dermal sensitisation has been published by Safford (2008, described in section 5.10.1). This proposal may, after refinement and validation, in the future be applicable for assessment of substances prior to incorporation into products, but is considered too limited for regulatory uses for the time being.

Regulatory use

The TTC concept is already being applied in different regulatory settings internationally, notably in the risk assessment of food contact materials, in the evaluation of flavouring substances in food and pesticide metabolites in ground water, and in the evaluation of genotoxic constituents in pharmaceuticals for humans and in herbal medicinal products and preparations.

The US-FDA permits the use of the general TTC concept, called Threshold of Regulation (TR), in the regulation of food packaging materials when the overall dietary concentration of an identified migrant of known chemical structure from packaging material is below 0.5 µg/kg food, which equals the TTC value of 1.5 µg/person/day (described in section 4.1.1). The US-FDA assumes a total daily intake of food and drink of 3 kg (1.5 kg of solid food and 1.5 kg of liquid food) for an adult person with a body weight of 60 kg. In this case no toxicity testing is required, although a toxicity profile based on available data is requested. Above this threshold, the degree of required testing increases as estimated exposure increases (Table 6 in section 4.1.1).
In the EU, the European Food Safety Authority (EFSA) has not formally used the TTC concept in the assessment of food contact materials. However, in the evaluation of migration of food contact materials the EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids uses thresholds to decide the amount of toxicity data needed to be supplied by the petitioner. As a general principle, the greater the exposure through migration, the more toxicological information will be required (see Table 7 in section 4.1.2).

The structure-based, tiered TTC approach as outlined by Munro et al. (1996, 1999) is used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in a procedure for the evaluation of flavouring substances in food (described in section 4.2.1). The EFSA also uses this approach for evaluation of flavouring substances (described in section 4.2.2), except that the general TTC value of 1.5 µg/person/day (0.025 µg/kg bw/day) is not accepted. The procedure takes into account available information on structure-activity relationships, metabolism, intake and toxicity data on groups of structurally related flavouring substances. The procedure for the safety evaluation of flavouring agents proceeds through a number of steps in which several questions have to be answered.

The TTC concept has been proposed in the EU in the assessment of certain metabolites of active substances of plant protection products in groundwater. A guidance document has set criteria for the conditions in which a metabolite is to be considered relevant. For metabolites considered to be not relevant, a TTC approach should be followed and a TTC value of 1.5 µg/person/day has been proposed. Assuming a consumption of 2 litres of water per day, this TTC value relates to an acceptable upper limit for the concentration of the metabolite in groundwater of 0.75 µg/l. This threshold is only considered acceptable if the metabolite in question has a lower biological activity than the parent compound, is not genotoxic, and is not classified as toxic, reprotoxic or carcinogenic.

The European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) has released a “Guideline on the Limits of Genotoxic Impurities” (described in section 4.4.1.1). For genotoxic compounds without sufficient evidence for a threshold-related mechanism, the Guideline proposes the application of a TTC concept to determine acceptable impurity levels with reference to the paper of Kroes et al. (2004). A TTC value of 1.5 µg/day (corresponding to an estimated 10^{-5} lifetime risk of cancer) was recommended as an acceptable limit for genotoxic impurities in drug substances, since it was considered that benefits of pharmaceuticals would justify a lifetime risk of cancer of 10^{-5}.

The EMA Committee on Herbal Medicinal Products/preparations (HMPC) has released a guideline on the assessment of genotoxicity of herbal medicinal products/preparations (described in section 4.4.2). The HMPC proposes to use the same TTC concept as described in the EMA/CHMP guideline on genotoxic impurities in medicinal products.

A number of potential applications of the TTC concept have been proposed in the risk assessment of chemicals for which there are no or little toxicological data, but the exposure is expected to be low, including industrial chemicals within the new EU chemical regulation REACH (Registration, Evaluation, and Authorization of CHemicals) (described in section 5.1), food additives and contaminants (described in section 5.2.1 and 5.2.2, respectively), veterinary drug residues in food of animal origin (described in section 5.3), drinking water contaminants and materials intended for use in contact with drinking water (described in section 5.4), and consumer products such as cosmetics and household care products (described in section 5.5 and 5.6, respectively).

Within REACH the generic TTC concept illustrated in Figure 5 (in section 5.1.1) could be applied for the chemical safety assessment for data poor substances at low tonnage levels as well as for substances where data do not allow for the derivation of the so-called 'Derived No Effect Level' (DNEL). Another area is in relation to exposure based waiving, where the documentation of an exposure level below a certain TTC level would make further testing superfluous. However, there is no reference to any threshold values in the guidance document for the implementation of REACH (ECHA 2008a). A promising proposal for exposure-triggered toxicity testing by using the TTC concept has been published by Bernauer et al. (2008, described in section 5.1.5) and demonstrated for fertility and developmental toxicity (described in section 5.10.2).
The expert group under the Nordic Council of Ministers has expressed a sceptical view about the use of the TTC concept in REACH due to limitations and uncertainties of the present approaches (NCM 2005, described in section 5.1.2).

The EU Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) have evaluated the appropriateness of the TTC concept for the human health risk assessment of chemical substances in their recently published draft opinion (DG SANCO 2008, described in section 3.7).
The DG SANCO Expert Group concluded that the principle of the TTC concept in itself is scientifically acceptable, but that the application in terms of risk assessment for safety evaluation of a chemical is dependent on the quality, quantity, and relevance of the underlying toxicity database, and a reliable estimation of the exposure to the chemical in the respective field of application.
The DG SANCO Expert Group noted that several classes of chemicals have been identified, for which the TTC concept cannot be applied and that the concept can presently not be applied for certain endpoints, like allergic reactions, intolerance, local effects and pharmacological effects. The DG SANCO Expert Group also noted that additional limitations exist with regard to extrapolation to other exposure routes (inhalation and dermal) and that recently published preliminary data on the RepDose database suggest that

there is some doubt about the classification system by Cramer and that refinements are needed.
Furthermore, the DG SANCO Expert Group emphasised the essentiality of appropriate exposure assessments for the application of the TTC concept. In the case of genotoxic impurities in pharmaceuticals and of food flavourings, the available information was considered adequate. However, limited knowledge exists in other areas, e.g. for consumer products, where a large diversity of products exists and complex exposure scenarios have to be considered including multiple exposure routes. In relation to cosmetic ingredients, the current database was considered inadequate.
Finally, the DG SANCO Expert Group stressed the need for further research in the development and validation of the current toxicity databases particularly in the areas where an insufficient number of representative chemicals is included. In addition, the methodology for assessing systemic exposure needs to be improved and appropriate data on exposure need to be generated for the various exposure scenarios.

Potential applications of the TTC concept in the Danish administration

It is recommended that the Danish Environmental Protection Agency (MST) and the Danish Veterinary and Food Administration (FVST) examine the possibilities for potential applications of the TTC concept in the future within specific areas.

A general area where the TTC concept may be useful would be in setting priorities for allocating resources for risk assessment, toxicological testing, development of analytical methodologies, and more refined exposure assessments by identifying those chemicals for which exposure estimates are below or above the relevant TTC values (described in section 7.1). In case of a significant exposure level above the relevant TTC value, priority could be allocated for a more detailed assessment and, if necessary, for obtaining further information.

Another area in which the TTC concept could be a valuable tool is as a preliminary, and sometimes only, step in a risk assessment of chemicals identified to be present at low concentrations in consumer products, food and environment, for which toxicity data are lacking, but for which exposure assessments can be undertaken. Thus, the TTC concept could be a valuable tool in a variety of specific cases where e.g., a very small amount of a chemical contaminant has been found in consumer products, food etc. and where the authorities by the use of the TTC concept would be able to respond quickly on any public concern and make quick priority for further risk management. Although the TTC concept is not designed to replace conventional approaches to risk characterisation for established and well-studied chemicals, such as pesticides and food additives, it may also be used to evaluate small amounts of unintended impurities and/or breakdown products of such compounds.

The TTC concept provided by Kroes et al. (2004, described in section 3.4), as amended by Felter et al. (2009, described in section 5.2.4) is recommended for substances where systemic effects are considered as being the critical effect(s).

Application of the TTC concept could be considered by the MST within the following areas (described in section 7.2):

- industrial chemicals, REACH
- drinking water contaminants and materials intended for contact with drinking water
- non-persistent contaminants in soil
- ambient air pollutants (at present only for systemic effects)
- personal care products, including cosmetics (some reservations, see section 7.2.5)
- consumer products, including household care products
- genotoxic impurities

The DTU National Food Institute already uses the TTC concept as provided by Kroes et al. (2004) in their advises to the FVST on the food safety resulting from the use of disinfectants for decontamination of surfaces in production plants that may potentially come into contact with food (described in section 7.3.2).
Application of the TTC concept could be considered by the FVST within the following areas (described in section 7.3.1):

- substances migrating from food contact materials
- veterinary drug residues
- non-persistent contaminants found in animal feed, its raw materials, or additives
- residues of pesticide metabolites
- some mycotoxins
- naturally occurring toxicants in food plants
- food contaminants originating from the environment or processing
- substances used as processing aids at low concentrations in a very limited number of food items.

Sammenfatning

Antagelse om at der er et niveau (tærskel) for eksponering for kemiske stoffer, under hvilken der ikke ses sundhedsskadelige effekter, og dermed ingen betydelige risici for påvirkning af menneskers sundhed, er vidt accepteret for de fleste typer af sundhedsskadelige effekter (endpoints). For stoffer, der både er genotoksiske og kræftfremkaldende, antages det, at effekter kan opstå selv ved meget lave eksponeringer, men risikoen for påvirkning af menneskers sundhed vurderes at være minimal. Af disse to antagelser er konceptet 'Threshold of Toxicological Concern' (TTC) opstået. TTC refererer således til en tærskel for human eksponering, under hvilken der vurderes ikke at være en nævneværdig risiko for påvirkning af menneskers sundhed selv ved en livslang eksponering for et givent kemisk stof.

Udviklingen af TTC konceptet er baseret på analyser af toksikologiske data og kemiske strukturer for en lang række forskellige kemiske stoffer. Konceptet er designet til brug som erstatning for stofspecifikke toksikologiske data i situationer, hvor der er begrænsede eller slet ingen data om et givent stof, og hvor eksponeringen af mennesker er ubetydelig eller så lav, at der ikke bør igangsættes nye toksicitetsundersøgelser af dyrevelfærdsmæssige såvel som af generelle ressourcemæssige årsager.

Den historiske udvikling af TTC konceptet

Hidtil er to forskellige tilgange blevet anvendt i TTC konceptet som udviklet til dato: Det generelle TTC koncept og et 'tiered' TTC koncept relateret til strukturelle oplysninger og/eller metaboliske og toksikologiske data for enkeltstoffer.

Det generelle TTC koncept, som også har til hensigt at omfatte kræftfremkaldende effekter, blev introduceret af Rulis (1986, 1989) fra den amerikanske Food and Drug Administration (US-FDA), som en 'Threshold of Regulation' (TR, beskrevet i afsnit 3.1). Rulis anvendte data for en delmængde på 343 stoffer, som alle var kræftfremkaldende i forsøgsdyr efter oral administration af stoffet og samlet i en database over kræftfremkaldende stoffer (Carcinogenic Potency Database, CPDB) (Gold et al. 1984). Baseret på analyser af disse stoffer foreslog Rulis oprindeligt, til illustration, en tærskelværdi på 0,15 µg/person/dag. Efterfølgende har Munro (1990) bekræftet forslaget fra Rulis og har i den oprindelige database inkluderet endnu flere stoffer, der er kræftfremkaldende i gnavere, hvilket bringer det samlede antal stoffer i CPDB op på 492 stoffer (Gold et al. 1989). Databasens robusthed er senere blevet bekræftet af Cheeseman et al. (1999), der udvidede data sættet i CPDB til 709 kræftfremkaldende stoffer baseret på løbende opdateringer af CPDB (beskrevet i afsnit 3.3). Munro (1990) konkluderede, at en tærskelværdi på 1,5 µg/person/dag vil give en høj grad af beskyttelse mod sundhedsskadelige effekter. Efterfølgende har US-FDA vedtaget denne tærskelværdi og fastsat en koncentration på 0,5 µg /kg fødevarer som en TR for stoffer, der anvendes i materialer og genstande beregnet til at komme i kontakt med fødevarer (Federal Register, 1993, 1995).

Munro et al. (1996) har undersøgt relationen mellem kemisk struktur og toksicitet af kemiske stoffer ved at lave en referencedatabase bestående af 613 kemiske stoffer, som er undersøgt for en række ikke-kræftfremkaldende toksikologiske endpoints i gnavere og kaniner i orale toksicitetsundersøgelser, herunder undersøgelser af sub-kronisk og kronisk toksicitet samt af reproduktions- og udviklingstoksicitet (beskrevet i afsnit 3.2). For mange af stofferne blev der identificeret mere end et enkelt NOEL (observeret nul-effekt niveau) og totalt indeholdt referencedatabasen på daværende tidspunkt 2941 NOEL værdier. Stofferne blev grupperet i en af tre potens klasser (klasse I, II og III) baseret på den kemiske struktur ved hjælp af et 'beslutningstræ' udviklet af Cramer et al. (1978) (beskrevet i afsnit 3.2). De kumulative fordelinger af logaritmerne til NOEL værdierne blev plottet særskilt for hver af de strukturelle klasser (figur 1 i afsnit 3.2). Fem percentilen for NOEL værdien blev estimeret for hver strukturel klasse og efterfølgende konverteret til en tærskelværdi for human eksponering under anvendelse af den konventionelle standard sikkerhedsfaktor på 100.
De struktur-baserede, 'tiered' TTC værdier blev fastsat til 1800 µg/person/dag for Cramer klasse I stoffer, 540 µg/person/dag for Cramer klasse II stoffer, og 90 µg/person/dag for Cramer klasse III stoffer (tabel 3 i afsnit 3.2). De endpoints, der er omfattet af TTC konceptet, inkluderer systemiske effekter bortset fra mutagenicitet og carcinogenicitet.
Efterfølgende er antallet af kemiske stoffer i databasen steget fra 613 til 900. Det skal bemærkes, at det udvidede antal stoffer ikke har betydet ændringer i de kumulative fordelinger af NOEL værdierne.

I senere analyser har Kroes et al. (2000, 2004) kombineret det generelle TTC koncept og det struktur-baserede, 'tiered' TTC koncept og udviklet et udvidet struktur-baseret, 'tiered' TTC koncept (beskrevet i afsnit 3.4).
Den TTC værdi på 1,5 µg/person/dag (0,025 mg /kg legemsvægt/dag), der anvendes af US-FDA som en TR, er blevet yderligere analyseret af Cheeseman et al. (1999), der ved brug af en udvidet database konkluderede, at der kan være nogle kemiske stoffer med en meget høj kræftfremkaldende potens, som ikke er omfattet af TTC konceptet (beskrevet i afsnit 3.3). Fem grupper af stoffer (tabel 4 i afsnit 3.4) blev identificeret, hvor en betydelig del af de inkluderede stoffer stadig kan give anledning til bekymring ved et indtag på 0,15 µg/person/dag (0,0025 mg/kg legemsvægt/dag), hvilket er en 10-faktor under TR værdi. Tre af disse grupper består af stoffer, som er genotoksiske: Aflatoksin-lignende stoffer samt azoxy- og ***N***-nitroso-forbindelser, mens to andre grupper indeholder stoffer, der er ikke-genotoksiske: 2,3,7,8-dibenzo-p-dioxin (TCDD) og dets analoger (dioxiner) samt steroider.

Stoffer, der har vist sig at skade nerve- og/eller immunsystemet samt fosterskadende stoffer blev også undersøgt nærmere (beskrevet i afsnit 3.4). Det blev konkluderet, at disse stoffer er omfattet af det struktur-baserede, 'tiered' TTC koncept bortset fra de nerveskadende stoffer, som tilhører gruppen af organophosphat pesticider. For sidstnævnte gruppe af stoffer blev TTC værdien sat til 18 µg/person/dag. Det skal understreges, at denne værdi for organophosphater ikke har til hensigt at erstatte de normale regulatoriske vurderinger for organophosphater, der anvendes som pesticider, men kan anvendes til at vurdere risikoen i tilfælde af, at et ikke-godkendt eller ureguleret organophosphat bliver fundet som en forurening i fødevarer.

TTC værdierne fastsat af Kroes et al. (2004) er præsenteret i tabellen efter dette afsnit (identisk med tabel 5 i afsnit 3.4). Som tidligere nævnt er aflatoxin-lignende stoffer, azoxy-forbindelser, ***N***-nitroso-forbindelser, 2,3,7,8-

dibenzo-p-dioxin (TCDD) og dets analoger, samt steroider ikke omfattet af TTC konceptet på grund af deres kræftfremkaldende potentiale. Da de databaser, som er blevet brugt til at udvikle TTC værdierne, ikke inkluderede toksicitetsdata for proteiner eller tungmetaller som f.eks. cadmium, bly og kviksølv, bør TTC konceptet heller ikke bruges ved vurderinger af sådanne stoffer. Da forbindelser med meget lange halveringstider og som viser meget store forskelle i bioakkumulering mellem dyrearter, som f.eks. TCDD og analoger heller ikke var med i den oprindelige database (Munro et al. 1996) er disse ligeledes ikke omfattet af TTC konceptet. Hertil kommer, at endokrint aktive kemiske stoffer heller ikke bør vurderes ved brug af TTC konceptet alene, da de tilgængelige data ikke er tilstrækkelige med henblik på, i lav-dosis området, at vurdere en klar årsagssammenhæng mellem hormon-lignende, endokrint aktive kemiske stoffer og sundhedsskadelige virkninger på mennesker.

TTC værdier (efter Kroes et al. 2004).

Stofgruppe	µg/person/dag	µg/kg lgv./dag
Genotoksiske stoffer	0.15	0.0025
Ikke-genotoksiske stoffer	1.5	0.025
Organophosphater	18	0.3
Cramer klasse III	90	1.5
Cramer klasse II	540	9
Cramer klasse I	1800	30

TTC konceptet som udviklet af Kroes et al. (2004) er for nylig blevet yderligere udviklet af Felter et al. (2009) til også at inkludere data fra Ames tests for kemiske stoffer med strukturelle indikationer for genotoksicitet samt at inkludere kortere varende eksponeringer (tabel 10 i afsnit 5.2.4).

På nuværende tidspunkt er TTC konceptet begrænset til oral eksponering, da databaserne bag konceptet ikke omfatter forsøg med dermal applikation eller eksponering ved indånding. Et lovende forsøg på at udvide TTC konceptet til også at omfatte inhalationseksponering er blevet fremsat af Escher et al. (2008a,b, 2010) baseret på analyser udført med RepDose databasen, som er udviklet på Fraunhofer Institute for Toxicology and Experimental Medicine (beskrevet i afsnit 5.9.2 og 5.9.4). Anvendelse af TTC konceptet for dermal eksponering har været diskuteret i forbindelse med ingredienser i kosmetik (beskrevet i afsnit 5.5.1). Disse forsøg på at udvide TTC konceptet til også at omfatte inhalation og dermal eksponering kan efter yderligere udvikling og validering i fremtiden muligvis anvendes til vurdering af kemiske stoffer i de tilfælde, hvor disse eksponeringsveje er relevante, men vurderes for tiden at være for lidt udviklede og validerede med henblik på regulatoriske anvendelser.

Da databaserne bag TTC konceptet udelukkende er baseret på systemiske effekter efter gentagen oral administration af de kemiske stoffer, er endpoints som akut toksicitet og lokale effekter som irritation og sensibilisering ikke omfattet af TTC konceptet. Den traditionelle tærskelværdi tilgang har generelt ikke været anvendt ved vurderinger i relation til allergiske effekter, hvorfor der generelt ikke er fastsat NOEL værdier baseret på allergi. Således er endpoints som allergi, overfølsomhed og intolerance heller ikke omfattet af TTC konceptet. Et forslag til anvendelse af TTC konceptet for dermal sensibilisering er blevet fremsat af Safford (2008, beskrevet i afsnit 5.10.1). Dette forslag kan, efter videre udvikling og validering, måske i fremtiden anvendes til stofspecifikke vurderinger, men vurderes for tiden at være for lidt udviklet og valideret med henblik på regulatoriske anvendelser.

Regulatoriske anvendelser af TTC konceptet

TTC konceptet har allerede forskellige regulatoriske anvendelser internationalt, nemlig inden for vurdering af materialer og genstande beregnet til kontakt med fødevarer, aromastoffer i fødevarer, pesticid metabolitter i grundvand, og genotoksiske urenheder i lægemidler til humant brug samt i naturlægemidler.

US-FDA tillader brugen af det generelle TTC koncept, kaldet Threshold of Regulation (TR), i reguleringen af emballager til fødevarer, når den totale koncentration fra fødevarer af en identificeret migrant fra fødevareemballager af kendt kemisk struktur er under 0,5 µg/kg fødevarer, som svarer til en TTC værdi på 1,5 µg/person/dag (beskrevet i afsnit 4.1.1). US-FDA antager, at det totale daglige indtag af mad og drikke er 3 kg (1,5 kg fast føde og 1,5 kg flydende føde) for en voksen person med en kropsvægt på 60 kg. I fald TR overholdes forlanges ingen toksicitetsundersøgelser, selv om en toksicitetsprofil baseret på tilgængelige data normalt er påkrævet for stoffer i emballager til fødevarer. Over TR stiger graden af den påkrævede testning i takt med at eksponeringen stiger (tabel 6 i afsnit 4.1.1).
I EU har Den Europæiske Fødevaresikkerhedsautoritet (EFSA) ikke formelt anvendt TTC konceptet ved vurderinger af materialer og genstande beregnet til kontakt med fødevarer. Men ved vurdering af migration af materialer og genstande beregnet til kontakt med fødevarer anvender EFSA's Ekspertpanel tærskelværdier til at beslutte hvilke toksicitetsdata, ansøgeren skal levere med henblik på godkendelse af et specifikt stof. Et generelt princip er, at jo større eksponering via migration, jo mere toksikologisk information kræves (se tabel 7 i afsnit 4.1.2).

Det struktur-baserede, 'tiered' TTC koncept udviklet af Munro et al. (1996, 1999) anvendes af Joint FAO/WHO Expert Committee on Food Additives (JECFA) ved vurderinger af aromastoffer i fødevarer (beskrevet i afsnit 4.2.1). EFSA anvender også dette koncept ved vurdering af aromastoffer (beskrevet i afsnit 4.2.2), bortset fra at den generelle TTC værdi på 1,5 µg/person/dag (0,025 mg/kg lgv./dag) ikke er accepteret af EFSA. Ved denne procedure tages der hensyn til de foreliggende oplysninger om struktur-aktivitets relationer, metabolisme, indtag og toksicitetsdata for grupper af strukturelt beslægtede aromastoffer. Proceduren for vurderingen af aromastoffer følger derefter en række trin, hvor flere spørgsmål skal besvares.

TTC konceptet er også blevet foreslået anvendt i EU i vurderingen af relevante metabolitter i grundvand af aktive stoffer i plantebeskyttelsesmidler. En vejledning har sat kriterier med henblik på vurdering af, hvornår en metabolit betragtes som relevant. For ikke-relevante metabolitter anbefales det at anvende TTC konceptet, og en TTC værdi på 1,5 µg/person/dag er blevet foreslået. Under antagelse af et indtag på 2 liter vand om dagen svarer denne TTC værdi til en acceptabel øvre grænse for koncentrationen af metabolitten i grundvandet på 0,75 µg/l. Denne tærskelværdi betragtes kun som acceptabel, hvis metabolitten har en lavere biologisk aktivitet end modersubstansen, ikke er genotoksisk, og ikke er klassificeret som toksisk, reproduktionstoksisk eller kræftfremkaldende.

Det Europæiske Lægemiddelagenturs (EMA) Komité for lægemidler til humant brug (CHMP) har udgivet en vejledning om grænser for genotoksiske urenheder i lægemidler til humant brug (beskrevet i afsnit 4.4.1.1). For genotoksiske stoffer, hvor der ikke er tilstrækkeligt bevis for en tærskelbaseret mekanisme, anbefales det i vejledningen at anvende TTC konceptet til

fastsættelse af et acceptabelt indhold af urenheder, idet der henvises til konceptet udviklet af Kroes et al. (2004). En TTC værdi på 1,5 µg/person/dag (svarende til en anslået 10^{-5} livstidsrisiko for udvikling af kræft) er anbefalet som en acceptabel grænse for indhold af genotoksiske urenheder i lægemidler til humant brug, idet det vurderes, at fordelene ved lægemidler berettiger en livstidsrisiko for kræft på 10^{-5} i forhold til den mere generelt anvendte livstidsrisiko for kræft på 10^{-6}.

EMAs Komité for naturlægemidler (HMPC) har udgivet en vejledning om vurdering af genotoksicitet i relation til naturlægemidler (beskrevet i afsnit 4.4.2). HMPC foreslår at anvende samme TTC koncept som beskrevet i EMA/CHMP vejledningen vedrørende genotoksisk urenheder i lægemidler til humant brug.

Udover de ovennævnte regulatoriske anvendelser af TTC konceptet er konceptet også blevet foreslået inden for andre områder i relation til risikovurdering af kemiske stoffer, for hvilke der ikke er nogen eller kun få toksikologiske data, men hvor eksponeringen forventes at være lav, herunder industrikemikalier reguleret i henhold til REACH (Registration, evaluering og godkendelse af kemikalier) (beskrevet i afsnit 5.1), tilsætningsstoffer og kontaminanter i fødevarer (som beskrevet i afsnit 5.2.1 og 5.2.2, henholdsvis), veterinære lægemiddelrester i fødevarer af animalsk oprindelse (beskrevet i afsnit 5,3), kontaminanter i drikkevand og materialer beregnet til kontakt med drikkevand (beskrevet i afsnit 5.4), og forbrugerprodukter som kosmetik og husholdningsartikler (beskrevet i afsnit 5,5 og 5,6, henholdsvis).

I relation til REACH kan det generiske TTC koncept illustreret i figur 5 (i afsnit 5.1.1) muligvis anvendes ved den såkaldte kemiske sikkerhedsvurdering af stoffer i lave tonnage niveauer, for hvilke der kun er få data samt for stoffer, hvor de tilgængelige data ikke kan danne baggrund for beregning af det såkaldte 'Derived No Effect Level' (DNEL).
Et andet område er i relation til vurdering af en lempelse af datakravet for stoffer, hvor dokumentation af et eksponeringsniveau under en vis TTC værdi vil betyde, at yderligere testning er overflødig. Det skal dog bemærkes, at der ingen henvisninger er til konkrete TTC værdier i det vejledende dokument for gennemførelsen af REACH (ECHA 2008a). Et lovende forslag til en sådan eksponeringsudløst toksicitetstestning ved brug af TTC konceptet er blevet fremsat af Bernauer et al. (2008, beskrevet i afsnit 5.1.5) og demonstreret for effekter på fertilitet samt udviklingstoksicitet (beskrevet i afsnit 5.10.2).
En ekspertgruppe under Nordisk Ministerråd har givet udtryk for skepsis vedrørende anvendelse af TTC konceptet i REACH på grund af de begrænsninger og usikkerheder, som ekspertgruppen har vurderet, at der er i det foreliggende koncept (NCM 2005, der er beskrevet i afsnit 5.1.2).

EU's Videnskabelige Komité for Forbrugerprodukter (SCCP), Den Videnskabelige Komité for Sundheds- og Miljørisici (SCHER) og Den Videnskabelige Komité for Nye og Nyligt Identificerede Sundhedsrisici (SCENIHR) har evalueret TTC konceptet med henblik på anvendelse ved risikovurdering af menneskers sundhed som følge af eksponering for kemiske stoffer (DG SANCO 2008, beskrevet i afsnit 3.7).
DG SANCO Ekspertgruppen konkluderede, at TTC konceptet i sig selv er videnskabeligt acceptabelt, men at anvendelsen ved risikovurdering af et specifikt kemisk stof afhænger af kvaliteten, mængden, og relevansen af de

tilgrundliggende toksicitet data, samt en pålidelig vurdering af eksponeringen for det kemiske stof inden for de respektive anvendelsesområder.
DG SANCO Ekspertgruppen bemærkede, at der er identificeret flere grupper af kemiske stoffer, hvor TTC konceptet ikke kan anvendes, ligesom at TTC konceptet i øjeblikket ikke kan anvendes for visse endpoints, såsom allergi, intolerance, lokale effekter (irritation og sensibilisering) og farmakologiske effekter.
DG SANCO Ekspertgruppen bemærkede også, at der foreligger yderligere begrænsninger med hensyn til ekstrapolation til andre eksponeringsveje (inhalation og dermal applikation), samt at nyligt offentliggjorte foreløbige analyser ved brug af RepDose databasen tyder på, at der kan sås tvivl om Cramer klassificeringssystemet, og at yderligere udvikling af konceptet derfor er nødvendigt.
Desuden understregede DG SANCO ekspertgruppen, at eksponeringsvurderingerne er essentielle for, at TTC konceptet kan anvendes for et givent kemisk stof. For genotoksiske urenheder i lægemidler til humant brug og for aromastoffer til fødevarer fandt man det foreliggende datagrundlag som værende tilstrækkeligt med henblik på anvendelse af TTC konceptet. Derimod fandt man, at der er begrænset viden inden for andre områder, som f.eks. forbrugerprodukter, hvor en stor mangfoldighed af produkter findes, og hvor komplekse eksponeringsscenarier skal vurderes, herunder flere eksponeringsveje. I relation til kosmetiske ingredienser fandt man det foreliggende datagrundlag som værende utilstrækkeligt med henblik på anvendelse af TTC konceptet.
Endelig understregede DG SANCO ekspertgruppen behovet for yderligere forskning med henblik på yderligere udvikling og validering af de nuværende tilgrundliggende databaser for TTC konceptet, især inden for de områder hvor et utilstrækkeligt antal repræsentative kemiske stoffer er inkluderet i databaserne. Desuden skal metoderne og modellerne til vurdering af systemisk eksponering forbedres, og relevante data for eksponering skal genereres for de forskellige mulige eksponeringsscenarier.

Mulige anvendelser af TTC konceptet af danske myndigheder

Det anbefales, at Miljøstyrelsen (MST) og Fødevarestyrelsen (FVST) vurderer mulighederne for fremover at anvende TTC konceptet inden for specifikke områder.

Et generelt område, hvor TTC konceptet kunne være nyttigt, er i relation til prioritering og allokering af ressourcer til risikovurdering, toksikologisk testning, udvikling af analytiske metoder, og bedre eksponeringsvurderinger, idet kemiske stoffer, hvor eksponeringsestimater er under eller over de relevante TTC værdier, kan identificeres (beskrevet i afsnit 7.1). I tilfælde af, at eksponeringen er betydeligt over den relevante TTC værdi, kunne man således prioritere, at der foretages en mere detaljeret vurdering og, om nødvendigt, prioritere at få yderligere oplysninger.

Et andet område, hvor TTC konceptet kunne være et værdifuldt værktøj, er som et foreløbigt, og i visse tilfælde det eneste skridt i en risikovurdering af kemiske stoffer, der er vist at være til stede i lave koncentrationer i forbrugerprodukter, fødevarer og miljø, og hvor der mangler toksicitetsdata, men en eksponeringsvurdering kan foretages. Således kunne TTC konceptet være et værdifuldt værktøj i en lang række konkrete tilfælde, hvor f.eks. en meget lille mængde af en kontaminant er fundet i forbrugerprodukter, fødevarer eller miljøet, og hvor myndighederne ved brug af TTC konceptet

ville være i stand til at reagere hurtigt med henblik på at prioritere yderligere risikohåndtering.
Selv om TTC konceptet ikke er designet til at erstatte de traditionelle tilgange til risikovurdering af kemiske, hvor der ifølge lovgivningen er generet mange data, som f.eks. ved godkendelse af pesticid aktivstoffer og tilsætningsstoffer, kunne konceptet dog anvendes til at vurdere små mængder af utilsigtede urenheder og/eller nedbrydningsprodukter af sådanne stoffer.

TTC konceptet, som udviklet af Kroes et al. (2004, beskrevet i afsnit 3.4), og yderligere udviklet af Felter et al. (2009, beskrevet i afsnit 5.2.4) anbefales således til brug ved risikovurderinger af kemiske stoffer, hvor systemiske effekter anses som værende de(n) kritiske effekt(er).

MST kunne således overveje at anvende TTC konceptet inden for følgende områder (beskrevet i afsnit 7.2):

- Industrikemikalier, REACH
- Kontaminanter i drikkevand samt materialer beregnet til at komme i kontakt med drikkevand
- Ikke-persistente kontaminanter i jord
- Kontaminanter i udeluften (p.t. kun for systemiske effekter)
- Personlige plejeprodukter, herunder kosmetik (visse forbehold, se afsnit 7.2.5)
- Forbrugerprodukter, herunder husholdningsartikler
- Genotoksiske urenheder

DTU Fødevareinstituttet anvender allerede TTC konceptet udviklet af Kroes et al. (2004) i rådgivningen af FVST om fødevaresikkerhed ved brug af desinfektionsmidler til rengøring af overflader i produktionsanlæg, som potentielt kan komme i kontakt med fødevarer (beskrevet i afsnit 7.3.2).
FVST kunne også overveje at anvende TTC konceptet inden for følgende områder (beskrevet i afsnit 7.3.1):

- Stoffer der migrerer fra materialer og genstande beregnet til at komme i kontakt med fødevarer
- Veterinære lægemiddelrester i fødevarer
- Ikke-persistente kontaminanter i foder, dets råvarer, eller tilsætningsstoffer
- Rester af pesticid metabolitter i fødevarer
- Visse mykotoksiner i fødevarer
- Naturligt forekommende giftstoffer i fødevareplanter
- Kontaminanter i fødevarer der hidrører fra miljøet eller ved forarbejdning af råvarer
- Stoffer der anvendes som tekniske hjælpestoffer i lave koncentrationer i et meget begrænset antal fødevarer

1 Introduction

Risk assessments for human health effects from exposure to non-carcinogenic xenobiotics are generally based on the concept that an exposure threshold exists for a given critical toxicological effect, i.e. there is an exposure (dose or concentration) below which no (adverse) effects are to be expected. This concept is widely accepted and used worldwide by regulatory bodies.

In the case of compounds that show both genotoxic and carcinogenic properties, i.e. the carcinogenic effect is initiated by damage of the genetic material following direct interaction of the compound with DNA, it is assumed that there may be a dose-dependent response at all doses above zero and thus, some risk is considered to exist at any exposure level. However, at low doses the risk may be immensely low and acceptable dose levels may be defined.

From this, the concept of Threshold of Toxicological Concern (TTC) has emerged, which refers to the establishment of a human exposure threshold value for chemicals, below which there would be no appreciable risk to human health assuming lifetime exposure. The establishment of various TTC approaches has been based on statistical analyses of toxicological data for a range of different and/or structurally-related substances, and extrapolation to levels considered being of negligible risk to human health.

A TTC concept might be used as a substitute for missing substance specific toxicological information in situations where the human exposure is judged to be so low that the undertaking of elaborate toxicity studies is considered inappropriate for reasons of manpower, cost and animal welfare.

Two principal approaches of TTC have been developed: The general TTC concept aimed at covering both carcinogenic and non-carcinogenic compounds, and tiered TTC approaches relating potential toxicological potency to chemical structural information.

This project was initiated in order to evaluate if and how a TTC concept can be applied for regulatory purposes by the Danish Environmental Protection Agency (MST) and the Danish Veterinary and Food Administration (FVST) instead of traditional risk assessments for human health effects of xenobiotics present in food or environment at low levels. The aim of this report is therefore to give a brief overview of the TTC approaches available, evaluate the scientific data behind the concepts including the uncertainties, and to address the regulatory applicability within the MST and FVST resort areas.

The basic principles for the traditional risk assessment process is briefly summarised in Chapter 2. The historical developments of the TTC concept and different TTC approaches are addressed in Chapter 3. The current applications of the TTC concept in the risk assessment of food contact materials, in the evaluation of flavouring substances in food and in the evaluation of genotoxic constituents in pharmaceuticals for humans and in herbal medicinal products and preparations are presented in Chapter 4 and further, potential applications within several other areas are outlined in

Chapter 5. Uncertainties, strengths and limitations of the TTC concept are discussed in Chapter 6 and various approaches for an improvement of the concept are addressed in this section as well. Potential uses in future regulations by FVST and MST of the TTC concept concerning human health aspects of xenobiotics present in food and the environment, respectively, are discussed in Chapter 7.

When comparing regulatory standards set by various national and international authorities and bodies, it should be noted that different body weights for an adult person (e.g., 60 kg or 70 kg) are used by these organisations in the conversion of daily dose levels expressed as for example mg/day to dose levels expressed in mass units as for example mg/kg body weight/day (mg/kg bw/day). The body weight will be stated in brackets whenever possible.

2 Risk assessment, principles

The basic principles for the traditional risk assessment process is briefly summarised in this chapter.

Risk assessment for human health effects generally entails a sequence of actions (Nielsen et al. 2008):

1) Hazard assessment, comprising

   a) Hazard identification: identification of the adverse effects, which a xenobiotic has an inherent capacity to cause, and

   b) Dose (concentration) - response (effects) assessment (hazard characterisation): estimation of the relationship between dose (or level of exposure) to a xenobiotic, and the incidence and severity of an effect, where appropriate.

2) Exposure assessment: estimation of the concentrations or doses of the xenobiotic to which human populations are or may be exposed. Exposure to xenobiotics can occur via different sources as e.g., food, drinking water, ambient air, consumer products, and the working environment.

3) Risk characterisation: estimation of the incidence and severity of the adverse effects likely to occur in a human population due to actual or predicted exposure to a xenobiotic, and may include "risk estimation", i.e., the quantification of that likelihood.

Exposure to a xenobiotic can result in a broad spectrum of effects varying from mild effects to fatal poisonings. The type and severity of the effects observed is generally correlated with the degree of exposure (dose concentration). The effects can be divided into two types:

1) Those effects, which are considered as having a threshold for the effect, i.e. a dose or exposure concentration below which the effect is not observed. This type of effects is often termed 'threshold effects'.

2) Those effects for which a threshold cannot be identified, e.g. carcinogenic effects, which are caused by damage following direct interaction of the compound with the genetic material. This type of effects is often termed 'non-threshold effects'. For these effects, it is assumed that there is a dose-dependent response at all doses above zero and thus, some risk is considered to exist at any exposure level.

Regarding the severity of a given effect, it is evaluated whether the effect can be considered as being 'adverse' or not. Generally, an effect is considered to be 'adverse' when there is a change in morphology, physiology, functional capacity, development, and/or life span in the exposed individuals, and when the incidence of the effect is statistically significantly different from that in the control group.

The hazard characterisation also involves an evaluation of the 'no observed adverse effect level' (NOAEL) and 'the lowest observed adverse effect level' (LOAEL) for the various effects observed.
The 'NOAEL' is defined as "The highest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism under defined conditions of exposure." Alterations of the above mentioned parameters may be detected, which are judged not to be adverse.
The 'LOAEL' is defined as "The lowest concentration or amount of a substance, found by experiment or observation, which causes an adverse alteration of morphology, functional capacity, growth, development or life span of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure."
It should be noted that the terms "NOAEL/NOEL" and "LOAEL/LOEL" often are used indiscriminately in the scientific literature and with various significances, i.e., without a distinction whether the 'no observed effect level' or 'lowest observed effect level' has been determined for an adverse effect or for a non-adverse effect. However, in most situations, the 'no observed effect level' or 'lowest observed effect level' could be interpreted as being determined for adverse effects regardless of the term used as the aim of the hazard assessment in general is to assess adverse effects. The term used in this report, is generally the term applied in the specific reference.

When all the relevant effect data (the toxicological data set) have been evaluated, the hazard(s) considered most important, 'the critical effect(s)', is identified, i.e., the effect(s), which is considered as being the essential one(s) for the risk characterisation.

For threshold effects, a NOAEL (or LOAEL) is identified for the critical effect(s), which may be used for the establishment of ADI/TDIs etc., e.g. by applying uncertainty factors, or taken forward to the risk characterisation and used in the Margin of Exposure (MOE) approach.
For non-threshold effects (e.g. carcinogenic effects, which are caused by damage of the genetic material), there is currently no clear consensus on an appropriate methodology for the estimation of a no-effect level.

The hazard assessment is generally based on data elucidating the toxicological effects in humans and experimental animals of a given xenobiotic.
Ideally, a complete data set including information on the toxicological effects such as acute and repeated dose toxicity, irritation, sensitisation, mutagenicity and genotoxicity, carcinogenicity, and toxicity to reproduction, as well as on toxicokinetics should be available for the hazard assessment. However, for many xenobiotics, a complete data set is generally not available.

3 The TTC concept, historical developments

The concept, that levels of exposure for chemicals can be defined below which, there are no significant risks to human health, is widely accepted, both for carcinogenic and non-carcinogenic endpoints. From this, the concept of the Threshold of Toxicological Concern (TTC) has arisen, which refers to the establishment of a human exposure threshold value for chemicals, below which there would be no appreciable risk to human health assuming lifetime exposure. The TTC concept is intended to be used as a substitute for missing substance specific toxicological information in situations where the human exposure is judged to be so low that the undertaking of elaborate toxicity studies is considered inappropriate for reasons of manpower, cost and animal welfare.

This section gives an overview of the different TTC approaches that have been suggested.

Two principal approaches have been used within the TTC concept developed to date:

1) The general TTC concept aimed at covering both carcinogenic and non-carcinogenic compounds

2) Tiered TTC approaches relating toxicological potency to chemical structural information.

3.1 General TTC concept (US-FDA)

Frawley (1967), in the context of regulation of food packaging materials, was probably the first to consider the possibility that there would be some uses of food-packaging materials which cannot involve any hazard to health of the consumer of food. He tried to develop a scientific basis for a start towards a "common-sense" approach to food-packaging regulation. He analysed a data set of non-tumourigenic end-points from 2-year, chronic toxicity studies on 220 chemicals given via the diet, which at that time represented about 90% of all available chronic toxicity studies. The chemicals were food additives, including colours, industrial chemicals, and compounds found in consumer products, including cosmetics, chemicals used in food packaging materials, and pesticides, and heavy metals. He divided the chemicals into 5 categories according to the dose at which no toxicological effects were observed, i.e. No Observed Effect Levels (NOELs), being <1, <10, <100, <1000 and <10,000 mg/kg in the diet, respectively, or else >10 000 mg/kg in the diet (Table 1). The 5 chemicals with NOELs below 1 mg/kg diet were all pesticides that were known either to accumulate in the body, or to (intentional) affect the function of the nervous system at low doses. Only 19 compounds had NOELs below 10 mg/kg diet, all of which were pesticides or heavy metals. Forty compounds had NOELs below 100 mg/kg diet of which 39 were pesticides or heavy metals, the remainder being acrylamide. The majority of the chemicals

(180/220) had NOELs above 100 mg/kg diet. From this analysis, Frawley (1967) concluded that if pesticides and heavy metals were excluded, only occasional (fewer than 1 out of 100) commercial compounds will have NOELs below 100 mg/kg diet and that an infinitely small number will exhibit any toxicity at 10 mg/kg diet or less. Consequently, he suggested that for food packaging chemicals, which were untested and of unknown toxicity, the level of 10 mg/kg diet should be selected as basis for the evaluation. The conventional 100-fold margin of safety was suggested applied to this level, giving a figure of 0.1 mg/kg in the total human diet, which was the dietary concentration for any food packaging chemical which he considered could be safely consumed by humans.
Frawley (1967) went on to consider the potential migrations of food contact materials into foods and concluded that "any component of an article contacting food which is present in the article itself or its coating at a level of 0.2% or less by weight will contribute to the diet at a level which can be of no possible public health significance".

Table 1. Distribution of no observed effect levels in 2-year chronic studies. (After Frawley 1967).

No-effect level (mg/kg diet)	All compounds (220)	Heavy metals and pesticides (88)	Others (132)
< 1	5	5	0
< 10	19	19	0
< 100	40	39	1
< 1000	101	72	29
< 10,000	151	86	65

Rulis (1986) conducted a similar analysis of the US-FDA's Priority-Based Assessment of Food Additives (PAFA) database containing 159 compounds with subchronic or chronic toxicity data and came to the same conclusion as Frawley (1967). Essentially, there was no risk of toxicity in rodents exposed to certain food additives at dietary levels less than 1 mg/kg bw/day, or in human terms, approximately 1 to 10 µg/kg bw/day, depending on the safety factor applied.

The general TTC concept, covering also carcinogenic effects of chemicals, was first introduced in 1986 by Rulis of the US Food and Drug Administration (US-FDA) as a 'Threshold of Regulation' (Rulis 1986).

The approach put forward by Rulis (1986) ***"…is based upon the premise that, through examination of a sufficiently large sample of toxicological data from both classical toxicological feeding studies and from carcinogenicity bioassays, some global delimiters of risk and exposure can be determined to define levels of human exposure and/or levels of migration of substances to food that can be said to fall below some "threshold of regulation". When this is the case, the substance in question would not necessarily need to undergo the rigors of the premarket safety evaluation requirements. Instead, the particular use of the substance could be accepted by the FDA after an abbreviated review of pertinent information, thus avoiding the need for the submission and agency approval of a food additive petition covering the use of the substance. Implicit in such a process is the absence of any indication that the substance in question is a carcinogen or other potent toxin. Known carcinogens would be subjected to more formal risk assessment and risk management decision-making."***

In the paper by Rulis (1986), data on a subset of 343 oral carcinogens from animal studies compiled in the Carcinogenic Potency Database (Gold et al. 1984) was used as an illustrative example.

In 1984, the Carcinogenic Potency Database (CPDB) included data on approximately 3000 long-term, chronic animal experiments with about 770 different chemicals. For each experiment a TD_{50} value was calculated. The definition given by Gold et al. (1984) of a TD_{50} value is: ***"For any particular sex, strain, species and set of experimental conditions, the TD_{50} is the dose rate in mg/kg bw/day that, if administered chronically for a standard period – the "standard lifespan" of the species – will halve the mortality-corrected estimate of the probability of remaining tumour-less throughout that period."***. This definition by Gold et al. is published in Peto et al. (1984).

Since then the database has been supplemented several times based on new studies becoming available (Gold et al. 1986, 1987, 1989, 1990, 1991, 1993, 1995, 1999, 2005) and the entire, updated database is available on the Internet (http://potency.berkeley.edu/).

By May 2010, it included analyses of the experimental designs for 6540 chronic, long-term animal cancer studies, including species, strain, route of administration, dose and protocol, on 1547 chemicals (CPDB 2010).

For the purpose of the discussion, Rulis (1986) defined the potency ***"…as the slope of a straight line connecting the point representing the TD_{50} … of Gold et al. (1984) with the point representing zero risk and zero dose".*** When the carcinogenic potencies were analysed by grouping them into ranges (excluding 2,3,7,8-tetrachlorodibenzo-***p***-dioxin and aflatoxin B1) and plotting them as a probabilistic distribution, they formed (on a semi-logarithmic scale) a curve that was Gaussian (normal) in shape. Then the distribution was transformed into an exposure distribution at a constant assumed risk of $1x10^{-6}$ per lifetime. This risk level was chosen because it was the upper bound level of risk identified by the US-FDA (Federal Register1985) as ***de minimis*** for the purpose of regulating the carcinogen methylene chloride. The resulting curve – termed a risk equivalent exposure distribution – describes the relative probability that a carcinogen selected at random from known carcinogens will be one that presents a risk of $1x10^{-6}$ per lifetime at the exposure level indicated on the horizontal axis.

According to Rulis (1986), the example illustrated that it can be predicted on a probabilistic basis that should a substance permitted under a threshold-of-regulation decision unknowingly be a carcinogen, it would, under the present analysis, have roughly a 60% chance of presenting greater than a $1x10^{-6}$ per lifetime level of risk at the 5 µg/kg food level of exposure, a dietary level that had been suggested as an appropriate level to use for deciding on a threshold for regulation. It was concluded by the author that: ***"It appears that there presently exists an adequate scientific basis of data and information on which to construct a threshold-of-regulation policy relating to food-contact substances."***

Initially, Rulis (1986, 1989) proposed, for illustration, a threshold value of 0.15 µg/person/day. Based on the distribution of the 10^{-6} risks from the CPDB, this value would intercept the distribution at the 85^{th} percentile, meaning that only 15% of carcinogens in the database would present a greater than $1x10^{-6}$ per lifetime risk at an intake of 0.15 µg/person/day.

Subsequently, a workshop reanalysed the Gold et al. (1984) database of 343 rodent carcinogens and confirmed the observation of Rulis (1986, 1989) that a dietary intake of 0.15 µg/person/day intercepted the distribution of the 10^{-6}

risks from the CPDB at the 85th percentile (Munro 1990). In addition, the workshop included more rodent carcinogens added to the original database, bringing the total to 492 rodent carcinogens (Gold et al. 1989). This reanalysis with a broader set of data produced essentially the same distribution of 10^{-6} risks as originally published (Rulis et al. 1986). The robustness of this distribution of the 10^{-6} risks based on carcinogenicity studies in rodents was confirmed by Cheeseman et al**.** (1999, described in section 3.3) who expanded the data set to 709 carcinogens based on the continuously updated CPDB database (Gold et al. 1984, 1986, 1987, 1989, 1990, 1991, 1993, 1995).

The workshop also noted (Munro 1990) that in the acceptance of any threshold values it was assumed that every new untested substance could be a carcinogen and could be as potent as the most potent 15% of carcinogens in the CPDB. Recognizing that not every new substance would turn out to be a carcinogen, the workshop constructed a table of risk avoidance probabilities (Table 2).

Table 2. Probability of a target risk not being exceeded at various threshold values. (After (Munro et al. 1999, as modified from Munro 1990).

Threshold value (µg/day	Percentage of chemicals presumed carcinogenic							
	100%	50%	20%	10%	100%	50%	20%	10%
	10^{-6} Target risk				10^{-5} Target risk			
0.15	86	93	97	99	96	98	99	>99
0.3	80	90	96	98	94	97	99	99
0.6	74	87	95	97	91	96	98	99
1.5	63	82	93	96	86	96	97	99
3	55	77	91	95	80	90	96	98
6	46	73	89	95	74	87	95	97

Table 2 shows the effect of various assumptions regarding the proportion of chemicals that are presumed carcinogens on the probability that a 10^{-6} risk standard will not be exceeded. It should be noted that as this proportion decreases, the probability of not exceeding a specific risk standard increases dramatically. Thus, for example, while there is a 63% chance that the risk will not exceed 10^{-6} with a value of 1.5 µg/person/day when 100% of new chemicals are assumed to be carcinogenic, the probability that the risk will be less than 10^{-6} is 96% when only 10% of new chemicals are assumed to be carcinogenic. Moreover, if one invokes a less conservative risk standard of 10^{-5} (Table 2, right), then the probability of not exceeding that risk at a threshold value of 1.5 µg/person/day exceeds 96% even if it is assumed that 50% of new chemicals are potential carcinogens. In theory, the probability of an untested substance having a potency greater than the median of the distribution of TD_{50}s from the CPDB (Gold et al. 1989) is 50%.

Rulis re-examined his previous selection criteria for a threshold value and those of Munro (1990) and concluded that a threshold value of 1.5 µg/person/day would provide a high degree of health protection and subsequently the US-FDA adopted this threshold value as the threshold of regulation (Federal Register, 1993, 1995). Thus, in 1993 the US-FDA proposed ***"…to establish a process for determining when the likelihood or extent of migration to food of a substance used in a food-contact article is so trivial as not to require regulation of the substance as a food additive"*** (Federal Register 1993). A dietary concentration of 0.5 µg/kg food was proposed as the threshold of regulation for substances used in food-contact articles. Assuming a daily consumption of food and drinks of 3 kg, this would equate to an intake of 1.5 µg/person per day. The rationale behind this value was that it was 2000 times

lower than the dietary concentration at which the vast majority of 220 studied compounds were likely to cause non-carcinogenic toxic effects, and 200 times lower than the chronic exposure level at which potent pesticides induced toxic effects. Furthermore, based on the range of potencies exhibited by 477 oral animal carcinogens selected from the CPDB (Gold et al. 1989), most known carcinogens posed less than $1x10^{-6}$ lifetime risk if present in the daily diet at 0.5 µg/kg food. The US-FDA noted that such an exposure level would result in a negligible risk even in the event that a substance of unknown toxicity was later shown to be a carcinogen.

The threshold of regulation approach has been applied by US-FDA to food contact materials since 1995 (Federal Register 1995) for substances that are not known to be carcinogens and that do not contain structural alerts indicative of carcinogenicity. Named substances of known chemical structure meeting the threshold of regulation criteria, i.e. whose use in food contact articles result in a dietary concentration of the substance of 0.5 µg/kg food (corresponding to an intake of 1.5 µg/person per day assuming the consumption of 3 kg g food and liquid per day) or less would not require toxicological testing. However, submission of a literature search on the substance is requested.

3.2 Structure-based, tiered TTC concept (Munro et al. 1996)

Already in 1982, the US Food and Drug Administration (US-FDA) in its "Redbook" (US-FDA 1982), which outlined requirements for testing of food and colour additives, coupled knowledge of exposure with information on chemical structure (and presumed structure activity relationships (SAR)) to rank chemicals into various concern levels. For this purpose, the US-FDA used, in part, the procedure proposed by Cramer et al. (1978) concerning presumed SAR. However, the structure-based tiered TTC concept was first introduced by Munro et al. in 1996 and has been modified and accepted by JECFA (WHO 1997, IPCS 1998) for the evaluation of flavouring substances used in food.

Munro et al. (1996) explored the relationship between chemical structure and toxicity through the compilation of a reference database consisting of 613 chemical substances tested for a variety of non-carcinogenic toxicological endpoints in rodents and rabbits in oral toxicity tests, including sub-chronic, chronic, reproductive and developmental toxicity. The reference database presented the toxicity in terms of no-observed-effect levels (NOELs) for a wide variety of organic chemicals of divergent structure. The substances were grouped into one of three general potency classes based on the chemical structure using the decision tree of Cramer et al. (1978). The structural classification was based on the assumption that inherent toxicity is dependent on chemical structure. For many of the substances, more than one NOEL was identified and in all, the reference database contained 2941 NOELs.

The classification by the decision tree method of Cramer et al. (1978) gave the following results: 137 (22%) of the substances fell into the presumptively least toxic class (Class I), 28 (5%) into the intermediate class (Class II) and 448 (73%) into the presumptively most toxic class (Class III). According to the authors, the fact that there are far more substances in Class III than in Class I or II, reflects partially the conservative nature of this method, but also reflects the fact that the substances that toxicologists or regulators have chosen for the rather extensive testing required to meet the criteria necessary for

inclusion in the reference database, were in many cases chosen at least in part of the basis of some suspicion arising from chemical structure.

The decision tree method of Cramer et al. (1978) was based on the toxicological data then available and uses a series of 33 questions, each leading either to another question or to classification into one of three classes of presumptive toxicity. The questions were primarily based on chemical structure, but natural occurrence in body tissues or fluids as well as natural occurrence in traditional foods was also considered. The three Cramer classes of substances were defined as follows:

- Class I substances are those with structures and related data suggesting a low order of oral toxicity. They have simple chemical structures and are efficiently metabolised by high-capacity pathways. If combined with low human exposure, they should enjoy an extremely low priority for investigation. The criteria for adequate evidence of safety would also be minimal.
- Class III substances are those that have chemical structures that permit no strong initial presumptions of safety, or that may even suggest significant toxicity. They thus deserve the highest priority for investigation. Particularly when per capita intake is high or a significant subsection of the population has a high intake, the implied hazard would then require the most extensive evidence for safety-in-use.
- Class II substances are simply 'intermediate' substances with less clearly innocuous structures than those of Class I substances, but without structural features suggestive of toxicity.

The functional groups that characterise the chemicals in Cramer's Class III, i.e. the substances with the highest potential for toxicity, are:

- 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 lactones or α,β-unsaturated lactones
- 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
- substances without a strong anionic group for every 20, or fewer, carbon atoms (for substances not classified at earlier steps)

The European Chemicals Bureau (ECB) has commissioned the development of a computer software program (toxTree) to encode the Cramer classification scheme. toxTree (Version 2.1.0) is available as a free download upon registration from the Ex-ECB website at http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/.

The reference database compiled by Munro et al. (1996) was used as a basis to derive a threshold of acceptable human exposure for each of the structural classes that could be applied in the absence of specific toxicity data on a

substance within one of the three structural classes. For each of the 613 substances, the most conservative NOEL was selected, based on the most sensitive species, sex and endpoint. Cumulative distributions of the logarithms of NOELs were plotted separately for each of the structural classes (Figure 1). There was a distinct separation of the cumulative distributions for each of the three structural classes, which according to the authors supported the contention that chemical structure defines toxicity.

The 5th percentile NOEL was estimated for each structural class and this was in turn converted to a human exposure threshold by applying the conventional default safety factor of 100. A 100-fold safety factor was considered by the authors to provide a reasonable margin of safety in translating the results of analysis of the reference database to humans. The human intake thresholds for non-carcinogenic effects obtained are presented in Table 3.

The authors recognised that the presented human exposure thresholds for non-carcinogenic effects (Table 3) were considerably higher than the intake of 1.5 µg/person/day as a threshold of regulation, which is used by the US-FDA. They therefore stressed that the presented human exposure thresholds were intended to apply to chemically defined substances for which there is no presumption of genotoxic carcinogenicity. Otherwise, the 1.5 µg/person/day threshold might be a more appropriate value.

Later work increased the number of chemicals in the database from 613 to 900 without altering the cumulative distributions of NOELs, adding further reassurance about the validity of using this database to derive the TTC values (Barlow 2005, described in section 3.4).

Figure 1. Empirical cumulative distribution of NOELs of compounds in the reference database (Munro et al. 1996) and log-normally fitted cumulative distributions (solid lines). Compound grouped into the structural Classes I (□), II (ÿ), and III (ÿ) of Cramer et al. (1978).

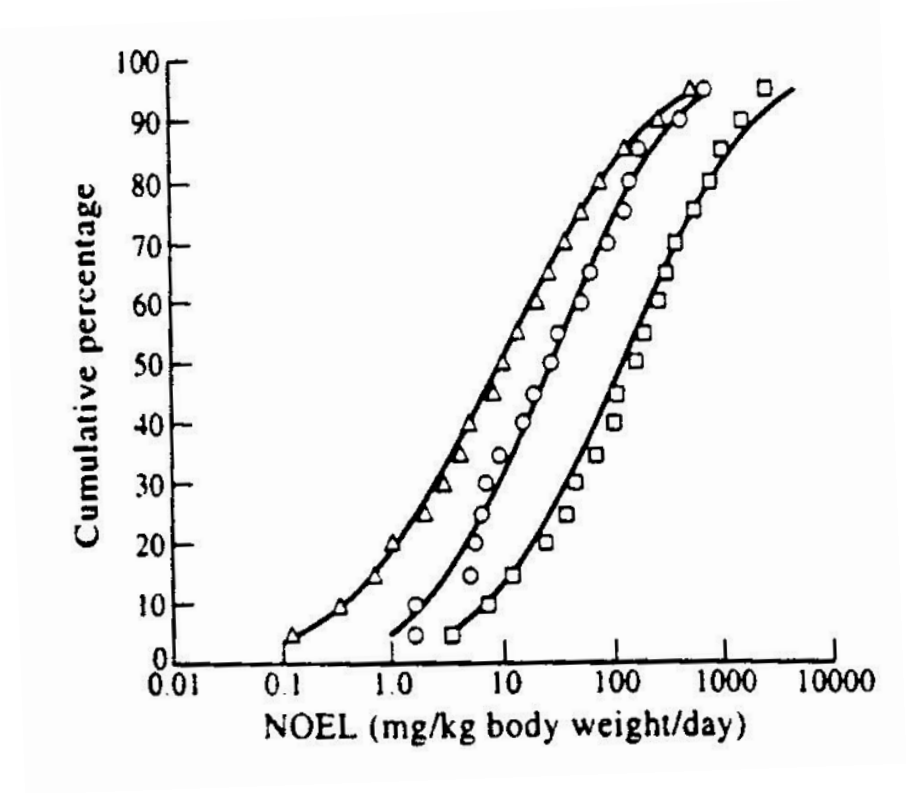


Table 3. Derivation of human exposure threshold (of toxicological concern) (TTC) values from toxicity data. (After Munro et al. 1996).

Cramer structural class	Fifth percentile NOEL (mg/kg bw/day)	Human exposure threshold (TTC) (mg/person/day)
I	3.0	1.8
II	0.91	0.54
III	0.15	0.09

Taken together, the structural TTC concept introduced by Munro et al. (1996) is based on a large database containing chemical structures and NOELs for sub-chronic, chronic, reproductive, and developmental oral toxicity for a wide variety of organic substances divided into three structural classes using the principles established by Cramer et al. (1978). Apart from structural information, no specific data are needed. Endpoints covered include systemic toxicity except mutagenicity and carcinogenicity. The TTC values are 1800 µg/person/day (Class I), 540 µg/person/day (Class II), and 90 µg/person/ day (Class III).

3.3 Extended US-FDA structure based, tiered Threshold of Regulation concept (Cheeseman et al. 1999)

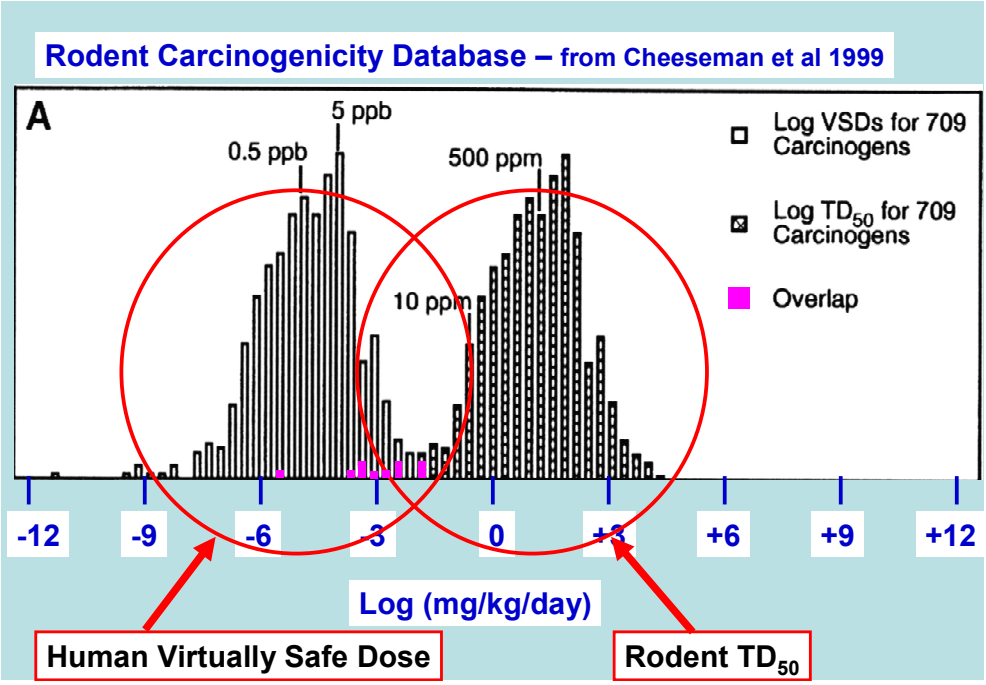
Cheeseman et al. (1999) further extended the Threshold of Regulation concept as adopted by US-FDA in 1995 (Federal Register 1995, described in section 3.1) by incorporation of acute and short-term toxicity data, the results of genotoxicity testing, and structural alerts to identify potent and non-potent carcinogens.

An evaluation of carcinogenic potencies was performed on 709 rodent carcinogens in the expanded carcinogenic potency database of Gold et al. (1995). Only compounds for which a TD_{50} for a given tumour type could be derived with a statistical significance of $p \leq 0.01$ or better on the basis of oral dosing studies were selected. In each case, the lowest TD_{50} was selected for the substance. Carcinogenic potencies were calculated based on the equation: Potency = $0.5/TD_{50}$ and, for comparison to previous data, the logarithm of the potencies were calculated and added the value 7 in order to create an adjusted log value (ALV) to ensure that all low values were greater than zero. Linear extrapolation to low dose was used to estimate a so-called virtually safe dose (VSD) corresponding to an upper-bound limit of lifetime cancer risk of one in a million. When these VSDs were plotted on a semi-logarithmic scale, they formed a normal distribution, with the mean potency corresponding to a VSD of approximately 1.2 µg/kg food in the daily diet (Figure 2).

The analysis shown in Figure 3.2 thus indicated that at the current dietary threshold of regulation of 0.5 µg/kg diet corresponding to a dietary intake of 1.5 µg/person/day, assuming a daily intake of 3 kg food and fluids, results in a risk estimate of more than 1 in a million for almost 50% of all known carcinogens.

In a next step, the carcinogens were divided into several subsets based on 1) the results on genotoxicity in the Ames assay (data for 442 of the 709 carcinogens), 2) structural alert classes for carcinogenicity, and 3) LD_{50}-values (i.e. the dose that causes death in 50% of the animals). To compare the distributions of the resulting subsets, the median adjusted log value (MALV) was calculated for the potencies of substances in each of the subsets as well as for the cohort of 709 substances.

Figure 2. Plots of the logarithms (added a value of 7) of the TD_{50} for 709 rodent carcinogens and the logarithms (added a value of 7) of the corresponding virtually safe doses (VSD). (After Cheeseman et al. 1999, kindly provided by Professor A. Renwick, Southampton, UK).



In order to identify structural alerts useful to support higher threshold levels, the most potent substances among the 709 carcinogens were examined. The structural alerts were correlated directly with the TD_{50}.

This work confirmed the validity of a threshold of regulation of 0.5 µg/kg diet, corresponding to a dietary intake of 1.5 µg/person/day assuming a daily intake of 3 kg food and fluids, as an appropriate threshold in cases where no specific toxicity data are available for a substance, and there is no reason based on the chemical structure (alerts) to suspect that the substance is a carcinogen. The range of potencies and likelihood of carcinogenicity associated with certain structural alerts or positive Ames test results suggested that they should be evaluated on a case-by-case basis. However, it was recommended that ***N***-nitroso or benzidine-like structural alerts, endocrine disruptors, hydrazines/triazenes/azides/azoxy compounds and strained hetero-nuclear ring structural alerts should not be the subject to threshold of regulation exemptions even at 0.5 µg/kg in the diet.

The results also indicated that a tiered threshold of regulation could be justified, using thresholds higher than 0.5 µg/kg diet (1.5 µg/person/day) for less potent carcinogens. Examination of the expanded database led to the conclusion that a dietary threshold of 4-5 µg/kg (12-15 µg/person/day) could be appropriate for substances without structural alerts, and even for substances with structural alerts, if they were negative in genotoxicity tests. The two exceptions to this were ***N***-nitroso and benzidine-like compounds, which should be excluded from regulation by threshold of regulation at all dietary concentrations. If substances had no structural alerts, were negative in tests for genotoxicity, and had LD_{50} values above 1000 mg/kg bw, a dietary threshold of regulation in the range of 10-15 µg/kg (30-45 µg/person/day)

could be possible. The actual threshold level would depend on the LD_{50} value for that particular substance.

In order to evaluate non-carcinogenic endpoints, Cheeseman et al. (1999) also analysed information from the Registry of Toxic Effects of Chemical Substances (RTECS) database on 3306 substances with oral reproductive toxicity data, and on 2542 substances for which there were data from other repeat-dose toxicity studies, in addition to the 709 carcinogens. The lowest low effect level (LLEL) was selected for each substance and a 1000-fold uncertainty factor was applied in order to establish a pseudo-acceptable daily intake (PADI). The most likely (median) value for the PADI was 8300-fold above the threshold value derived from the carcinogenic potency database. The results therefore supported the contention that a "virtually safe dose" based on carcinogenicity data would also protect against other toxic effects.

Based on the results of the above analyses, Cheeseman et al. (1999) suggested the following tiered TTC approach in which structural alerts, genotoxicity test results and short-term toxicity data could be used to extend the US-FDA's existing threshold of regulation approach:

- Threshold of Regulation: 0.5 µg/kg diet (TTC value: 1.5 µg/person/day):
  General threshold in cases where no specific toxicity test data are available for a substance and there is no reason, based on the chemical structure, to suspect that the substance is a carcinogen. Substances possessing positive Ames test results or certain structure alerts such as e.g. ***N***-nitroso or benzidine-like chemicals should be evaluated on a case-by-case basis.

- Threshold of Regulation: 5 µg/kg diet (TTC value: 15 µg/person/day):
  Threshold for chemicals without structural alerts for carcinogenicity regardless of the results of genotoxicity testing (Ames test), or substances testing negative in genotoxicity testing (Ames test).

- Threshold of Regulation: 15 µg/kg diet (TTC value: 45 µg/person/day):
  Threshold for chemicals without structural alerts for carcinogenicity regardless of the results of genotoxicity testing (Ames test), or substances testing negative in genotoxicity testing (Ames test) provided that the LD_{50}-value of the substances in an appropriate acute toxicity study is above 1000 mg/kg bw.

Taken together, the enhanced structural tiered TTC concept introduced by Cheeseman et al. (1999) is based on a large database for a wide variety of organic substances, including chemical carcinogens. Apart from structural information, genotoxicity (Ames test) and acute toxicity (LD_{50}-values), no specific data are needed. Endpoints covered include systemic toxicity. The TTC values are 1.5, 15 and 45 µg/person/day.

This tiered approach proposed by Cheeseman et al. (1999) has not been adopted by the US-FDA.

3.4 ILSI Europe enhanced structure based, tiered TTC concept (Kroes et al. 2000, 2004; Barlow 2005)

ILSI Europe (International Life Sciences Institute) established an expert group on TTC in 1996. In the discussion of the TTC concept, concerns had been raised whether the human exposure thresholds of 0.09, 0.54, and 1.8 mg/person/day suggested by Munro et al. (1996, described in section 3.2) for Cramer Class III, II, and I substances, respectively, would also cover potentially sensitive toxicological effects that might occur at low doses. Such effects would be effects on the reproductive system, nervous system, immune system, and endocrine system, especially during the development. Although the original database published by Munro et al. (1996) included some studies measuring these endpoints, they were considered insufficient in number to provide an answer to this question. The ILSI group therefore examined the possibility of defining a TTC value for chemical substances present in the diet for general toxicity, including carcinogenicity, as well as for specific endpoints, namely neurotoxicity and developmental neurotoxicity, developmental toxicity, and immunotoxicity. Endocrine toxicity and allergenicity were also addressed as two separate cases, using different approaches and methodology. The expert group also examined whether the TTC value of 1.5 µg/person/day adequately covers non-cancer toxicological endpoints. The initial work of the expert group was presented by Kroes et al. (2000).

Expanded databases were developed for the specific endpoints: neurotoxicity (82 substances, of which 45 had sub-chronic and chronic neurotoxicity data, and 37 had acute neurotoxicity data), immunotoxicity (37 substances), developmental neurotoxicity (52 substances) and developmental toxicity (81 substances). They were analysed to see whether these endpoints were more sensitive than those for structural Class III compounds in the original database of Munro et al (1996), and to see whether the TTC value of 1.5 µg/person/day derived from the carcinogenic potency database adequately covered such endpoints. The distributions of NOELs for the specific endpoints were compared with the distribution of NOELs for non-specific carcinogenic endpoints.

The main conclusions of the expert group are enclosed below (Kroes et al. 2000):

- The cumulative distributions of the NOELs for developmental toxicity, including developmental neurotoxicity, did not differ greatly from the cumulative distribution of NOELs for chronic toxicity of Class III chemicals as described by Munro et al. (1996), i.e. were not more sensitive than other non-specific endpoints.
- In the case of neurotoxicity, the distribution was almost one order of magnitude lower than the distribution of NOELs for chronic toxicity of Class III chemicals. However, all substances were accommodated within the TTC value of 1.5 µg/person/day.
- The database for immunotoxicity was too limited to draw a distribution of the NOELs and the immunotoxicity endpoint was evaluated by comparing immune NOELs with non-immune NOELs. The NOELs for immunotoxicity did not differ from the NOELs for the non-immunotoxicity endpoints.

- For the evaluation of endocrine toxicity, the data available did not permit the establishment of a clear causal link between endocrine active chemicals and adverse effects in humans.
- The allergenicity endpoint was not analysed as such as the traditional threshold approach has never been applied to food allergy, nor has a NOEL based on allergy ever been established. More data are necessary to determine threshold doses for food allergens.
- A TTC value of 1.5 µg/person/day provides adequate safety assurance and that chemicals present in the diet that are consumed at levels below this threshold pose no appreciable risk. However, it was also stated that the TTC value can never offer an absolute guarantee of safety but that it seems to be soundly based with respect to general toxicity and the particular endpoints examined. Endpoints for which validated methods had yet to be developed are e.g. endocrine activity and allergenicity.

In a subsequent analysis (Kroes et al. 2004), the ILSI expert group addressed a number of further questions regarding the application of the TTC concept. Consideration was given to provide 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 concept. It was also evaluated whether a separate consideration of metabolism and accumulation was necessary in the application of a TTC concept. The main conclusions of the expert group were (Kroes et al. 2004, Barlow 2005):

The TTC value of 1.5 µg/person/day (0.025 µg/kg bw/day), used by US-FDA in the Threshold of Regulation policy, is designed to protect against the toxicity of most chemicals, including those of unknown toxicity should they turn out to be carcinogenic. Nevertheless, there may be some chemicals with a very high carcinogenic potency that may be not covered by the Threshold of Regulation approach. The carcinogenic potency database used by Cheeseman et al. (1999) earlier, comprising 709 compounds, was further expanded to 730 compounds, and analysed in order to identify structural alerts that would give the highest calculated risks if present at very low concentrations in the diet. The structural groups are outlined in Table 4.

Table 4. Structural alert groups for carcinogenicity examined by Kroes et al. (2004).

Structural Group	Number of compounds in group
Aflatoxin-like compounds	5
Aromatic amines	162
Aromatic nitrates	33
Azo compounds	18
Azoxy compounds	5
Benzidine derivatives	14
Carbamates	20
Heavy metal containing compounds	7
Highly chlorinated compounds	54
Hydrazines	57
Miscellaneous Ashby alerts	41
-Nitro furyl compounds	34

***N*-Nitroso compounds**	105
Organophosphorus compounds	17
Steroids	11
Strained rings	15
Tetrahalogenated dibenzodioxins and dibenzofurans	5
Vinyl containing compounds	40

Five groups of compounds were identified having a significant fraction of their members that may still be of concern at an intake of 0.15 µg/person/day (0.0025 µg/kg bw/day), which is 10-fold below the Threshold of Regulation figure. Three of these groups are genotoxic; the aflatoxin-like, azoxy- and ***N***-nitroso-compounds, while two groups were non-genotoxic; 2,3,7,8-dibenzo-***p***-dioxin (TCDD) and its analogues, and the steroids. The ILSI Europe Expert Group concluded that compounds with these structural alerts for high carcinogenic potency require compound-specific toxicity data and should be excluded from any TTC approach. A TTC value of 0.15 µg/person/day could be used for all other substances with structural alerts for genotoxicity.

Specific considerations of metabolism and accumulation are not necessary in the application of a TTC concept provided that the substances are not likely to show very large species differences in accumulations such as e.g. polyhalogenated-dibenzo-***p***-dioxins, -dibenzofurans, and -biphenyls and related compounds, as well as non-essential heavy metals in elemental, ionic or organic forms. They are known to accumulate in the body, and the employed safety factors may not be high enough to account for species differences in rates of elimination of such chemicals. In addition, such chemicals were not included in the original database of Munro et al. (1996, described in section 3.2), on which the TTC concept is based. For heavy metals and dioxin-like compounds extensive knowledge of their toxicological effects already exists, making use of the TTC concept unnecessary.

Neurotoxicants were further explored. From plotting the NOELs for cholinesterase-inhibition by the most potent neurotoxicant, the organophosphates, separately from the other neurotoxicants, it was noted that the 5th percentile NOELs for organophosphates were lower, by about an order of magnitude, than the corresponding NOELs for other neurotoxicants, which were adequately covered by the Cramer Class III threshold. By applying a safety factor of 100 to the fifth percentile NOELs for organophosphates, a human exposure threshold of 18 µg/person/day was derived (Table 5). This threshold for organophosphates was not intended to replace the normal regulatory assessments and controls for organophosphates used as pesticides, but could be used to evaluate the risk should a non-approved or unregulated organophosphate be detected as a contaminant in food.

In certain regulatory approaches, an additional assessment factor (of up to 10-fold) is used for teratogenicity and it might be argued that teratogens should be considered as a separate class when applying the TTC concept (see also section 5.10.2).
Using data from studies on 38 known teratogens, the NOAEL of teratogenicity (T) was compared to the most sensitive NOAEL for embryotoxicity (E) endpoints (van Schothorst and Piersma 2003). An E/T ratio of each compound was determined by dividing the NOAEL for embryotoxicity by the NOAEL for teratogenicity. An E/T ratio higher than 1 would reflect the extent to which teratogenicity occurred at lower doses than

embryotoxicity. For eight compounds the E/T ratio was higher than 1. These compounds were either dioxins or genotoxic carcinogens and would therefore all be excluded from the TTC concept very early in the decision tree (Figure 3) anyway.
The ILSI Europe Expert Group therefore decided that the application of an additional assessment factor for teratogenicity is not needed in the application of the TTC concept and that a separate TTC class for teratogenic effects would not be necessary. The TTC values for Cramer Class I, II, and III would therefore adequately take care of teratogens.

The ILSI Europe Expert Group also considered that, because it is still not known whether endocrine disrupters, i.e., chemicals that directly or indirectly affect either the structure and/or the function of the hormone producing glands or the parts of the brain that control them, are active at very low exposures, it would be premature to include low-dose, endocrine-mediated effects in the TTC concept.
The issue of low-dose-effects of compounds with endocrine activity still gives rise to extensive debate, partly caused by the absence of reproducibility of reported low dose effects in experimental animal studies. In the view of many toxicologists exposures to endocrine active compounds are handled by the body primarily with adaptive homeostatic mechanisms. Only if the body is unable to regulate exposures within its limits of homeostasis the threshold of adversity can be crossed through overloading of an endocrine mechanism. This sequence of events is often referred to as endocrine disruption.

The ILSI Europe Expert Group also considered that whilst thresholds undoubtedly exist for sensitization and elicitation of allergic responses, they have not been established yet even for common allergens, and are known to vary between individuals and within an individual over time. Thus, although the TTC concept does take account for substances causing immunotoxicity other than allergenicity, it can not be used to assess the concern for allergenicity. Allergic risks should be controlled by other means, e.g. labelling. In addition, proteins should be excluded from the TTC concept because of their potential for allergenicity and because some peptides have potent biological activities and because they were not included in the original database.

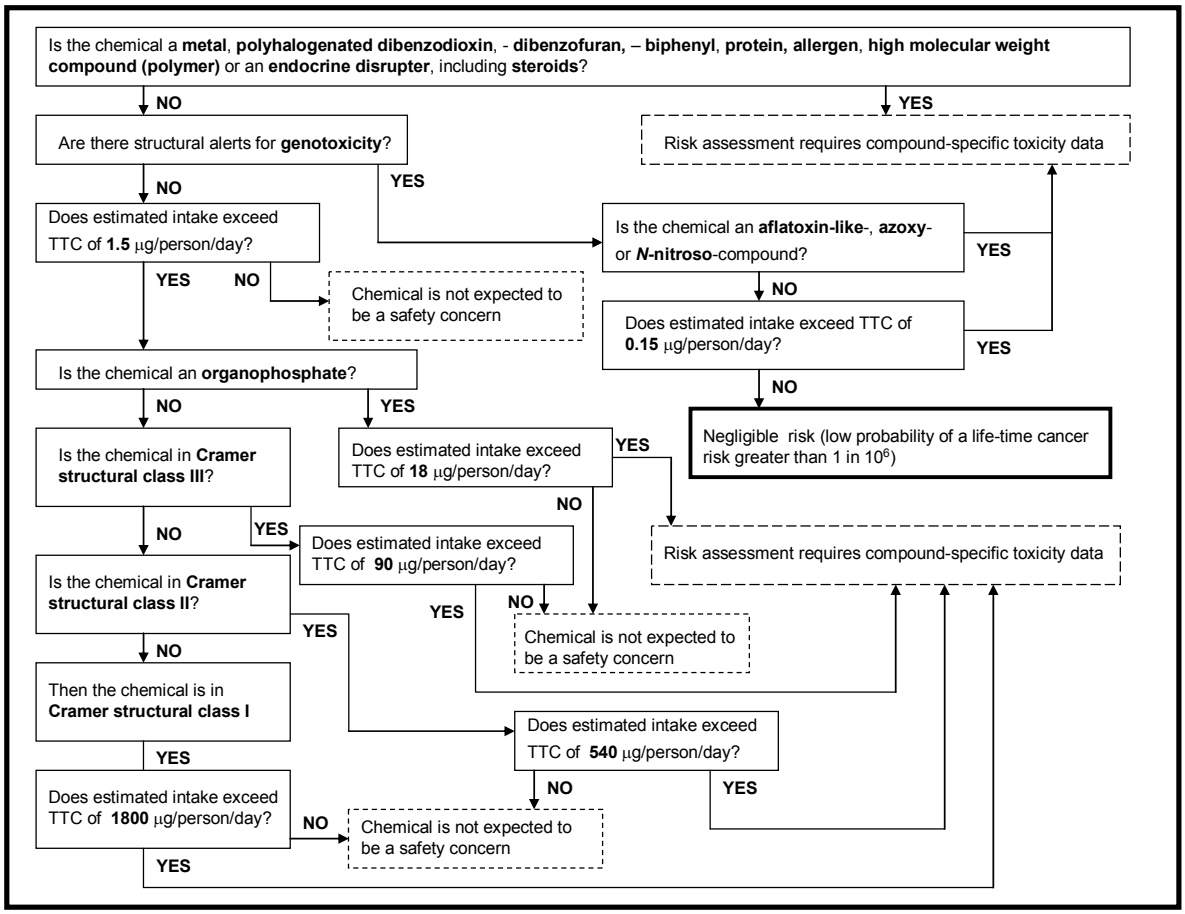
A decision tree incorporating a tiered approach was developed as guidance on how and when the TTC concept could be applied as a preliminary step in food safety evaluation (Figure 3). Proteins, heavy metals, and polyhalogenated dibenzodioxins and related compounds were excluded from the approach; for such substances, the risk assessment requires compound-specific toxicity data.

The initial step in the decision tree is the identification and evaluation of possible genotoxic and/or high potency carcinogens, i.e. aflatoxin-like compounds, ***N***-nitroso-compounds, and azoxy-compounds. For structural alerts that raise concern for potential genotoxicity, which are not among the high potency carcinogen alerts, a TTC value of 0.15 µg/person/day was proposed. Following this step, compounds that do not have structural alerts for genotoxicity are evaluated in a sequence of steps related to the concerns that would be associated with increasing intakes. For organophosphates, a TTC value of 18 µg/person/day was proposed. When the compound is not an organophosphate, the TTC values for the Cramer et al. (1978) structural

Classes III, II and I, with their respective TTC values (90, 540 and 1800 μg/person/ day, respectively) would be applied sequentially.

The decision tree was finalised following a peer review workshop held in March 2003, where the science behind the various steps in the tiered approach was presented and critically discussed. For any chemical taken through the decision tree process, one of two recommendations will be reached: either, the substance would not be expected to be a safety concern, or, risk assessment requires compound-specific toxicity data.

Figure 3. The TTC decision tree suggested by the ILSI Europe Expert group. (As modified from Barlow (2005) by the VKM 2006).



The decision tree is only applicable to chemicals of known structure, and with low molecular mass, as presented in the databases. A good estimate of intake or exposure is critical to the use of the decision tree, since this determines whether or not the TTC value is exceeded. The human exposure threshold (of toxicological concern) (TTC) values suggested by Kroes et al. (2004) to be used in the TTC decision tree for individual types of chemicals are summarized in Table 5.

Table 5. Human exposure threshold (of toxicological concern) (TTC) values. (After Kroes et al. 2004).

Type of chemical	µg/person/day	µg/kg bw/day
Genotoxic compounds	0.15	0.0025
Non-genotoxic compounds	1.5	0.025
Organophosphates	18	0.3
Cramer Class III	90	1.5
Cramer Class II	540	9
Cramer Class I	1800	30

In subsequent publications by Kroes et al. (2007) and Munro et al. (2008) a modification of the Cramer Class III threshold in the Kroes et al. (2004) decision tree was suggested. When the organophosphate compounds for which the additional TTC value of 18 µg/person/day was established by Kroes et al. (2004) these compounds were not excluded from the database behind the Cramer Class III threshold. When that was done a corrected Cramer Class III threshold of 180 µg/person/day instead of 90 µg/person/day could be established.

Taken together, the enhanced structural tiered TTC concept introduced by ILSI (Kroes et al. 2004) is based on a large database for a wide variety of organic substances, including chemical carcinogens. Apart from structural information, bioaccumulation potential, genotoxicity and carcinogenicity, no specific data are needed. Endpoints covered include systemic toxicity, including neurotoxicity and immunotoxicity. The TTC values are 0.15, 18, 90 (180), 540 and 1800 µg/person/day; for details, see above.

Although the TTC concept does take account for substances causing immunotoxicity other than allergenicity, it can not be used to assess the concern for allergenicity. In addition, proteins should be excluded from the TTC concept because of their potential for allergenicity and because some peptides have potent biological activities, and because they were not included in the original database.

3.5 General view on the TTC concept by the European Commission Scientific Steering Committee (2000, 2003)

In its first report from 2000 on the harmonisation of risk assessment procedures (EC-SSC 2000), the Scientific Steering Committee's Working Group on Harmonisation of Risk Assessment procedures in the Scientific Committees advising the European Commission in the area of human and environmental health expressed the view that "***the demand for the demonstration of the safety of an ever widening group of both natural and synthetic chemicals will require a reliable means of assessing priorities, and that this may be supported by use of the TTC approach. On this basis, provided the exposure level to a chemical is below the TTC value, it can be regarded as having no appreciable risk even in the absence of any toxicological data. In practise, it is important to have some additional reassurance by checking that the chemical structure does not indicate the***

potential for potent irreversible or serious toxic effects, i.e. there are no structural alerts." Although "the TTC concept is widely accepted by toxicologists, there is an ongoing debate about the actual level at which the TTC value should be set. Therefore, the Scientific Committees should address the concept of TTC and identify guidelines as to how it should be applied".

In the second report adopted in 2003 (EC-SSC 2003), the same committee described an attempt to establish a TTC concept and concluded

"***for chemicals where exposure levels are likely to be consistently low a staged approach to their risk assessment could be adopted:***

Stage 1: ***Examination of the chemical and physical properties to ensure that there are no structural alerts that could indicate a particularly high potency and therefore a need to treat the chemical differently.***

Stage 2: ***Evaluation of the likely worst case, total exposure when the chemical is in use. This should take into account exposure to other closely related chemicals. If the exposure levels are below the TTC, no new toxicological studies would be required.***

Stage 3: ***If the exposure levels are only just below or within an order of magnitude above the TTC value limited toxicological testing would be required concentrating on the potential to cause specific effects, e.g. genotoxicity. At this stage, in principle, in vitro tests could have a major role.***

Stage 4: ***Full hazard characterisation. This would only be needed for those chemicals that raised important concerns during stages 1-3.***

It should be noted that both the selection of an appropriate TTC value and the reliability of the structural alert scheme are dependent on a very robust and comprehensive database. Adoption of a TTC approach would be in line with the aim of the Commission of reducing animal use for testing purposes and avoiding unnecessary costs to industry. It would, however, place much more reliance on the development of reliable means of exposure assessment and provide great assistance in priority setting of chemicals for risk assessment."

3.6 Tiered TTC concept proposed by ECETOC (2004)

ECETOC (2004) has proposed a concept of generic threshold values based on hazard categories primarily intended to be used in the risk assessment procedure of industrial chemicals within REACH. The hazard categories are based on classification limits and for each substance to be risk assessed, inclusion in hazard categories depends on the substance's specific classification (or no classification) according to the Commission Directive 67/548/EC (EEC 1967). Three hazard categories have been suggested:

1. Low hazard category:
   - Substances classified as harmful (Xn;R20/21/22) for acute toxicity, and
   - Substances classified as irritating to eyes, respiratory system and skin (Xi;R36/37/38).

2. Medium hazard category:
   - Substances classified as toxic (T;R23/24/25) for acute toxicity,

- Substances classified as harmful (Xn;R48/20/21/22) for repeated dose toxicity,
- Substances classified as carcinogens, mutagens and reproductive toxins in category 3,
- Substances classified as skin sensitizers (Xi;R43), and
- Substances classified as severe eye irritants (Xi;R41)

3. High hazard category:
   - Substances classified as very toxic (Tx;R26/27/28) for acute toxicity,
   - Substances classified as toxic (T;R48/23/24/25) for repeated dose toxicity,
   - Substances classified as carcinogens, mutagens and reproductive toxins in category 1 and 2, and
   - Substances classified as respiratory sensitizers (Xi;R42).

Generic Exposure Values (GEVs) are generic threshold values for occupational inhalational and dermal exposure derived from OELs (Occupational Exposure Limits). The effects used to estimate GEVs are acute and repeated dose toxicity for a total of 63 organic and non-organic substances, both volatile and non-volatile.
For inhalation of solids, the GEVs are: 0.005, 0.1 and 1 mg/m^3, while for inhalation of volatiles, the GEVs are 0.05, 1 or 10 ppm for high, medium and low hazard category, respectively.
A margin of exposure of 2 has been selected as a basis for distinguishing scenarios that are of concern from those which are unlikely to be of concern. Consequently, for inhalation of solids, the GEVs are corresponding to an intake of 25, 500 and 5000 µg/person/day for high, medium and low hazard category, respectively, based on a respiratory volume of 10 m^3/day.
It should be noted that, in addition to the scientific toxicological information, the OELs include socio-economic and technical arguments.

Generic Lowest Exposure Values (GLEVs) are further suggested to be used for oral, inhalational and dermal exposure in tiered processes of consumer risk assessment as an estimate of the actual LOAEL for the substance's repeated dose toxicity. The GLEVs are based on the classification limit (50 mg/kg bw/day; R48 "Danger of serious damage to health by prolonged exposure"; based on a 90-day study) for repeated dose toxicity according to the Commission Directive 67/548/EC (EEC 1967). None of the values includes carcinogens, mutagens and reproductive toxins in category 1 and 2.
An assessment factor of 240 is applied to take into consideration: extrapolation from LOAEL to NOAEL (a factor of 6), extrapolation from sub-chronic to chronic study (a factor of 2), and inter- and intraspecies variation (a factor of 4 and 5, respectively).
For oral intake, the GLEVs are 0.5, 5, and 50 mg/kg bw/day, corresponding to an intake of 150, 1500, and 15000 µg/person/day for high, medium and low hazard category, respectively, for a 70 kg person.
For inhalation of solids, the GLEVs are 2.5, 25, and 250 mg/m^3, corresponding to an intake of 210, 2100, and 21000 µg/person/day for high, medium and low hazard category, respectively, based on a respiratory volume of 20 m^3/day.

Taken together, the tiered TTC concept introduced by ECETOC (2004) is based on a database for a wide variety of organic substances (GEVs/GLEVS), and for GEVs also in-organic substances, both volatile and non-volatile. Basic

data requirements are the EU classification of the substance. Endpoints covered include acute (GEVs only) and repeated dose toxicity. Category 1 and 2 carcinogens, mutagens and reproductive toxins are not covered. Regarding TTC values, see above.

3.7 Opinion on the TTC concept by the EU Scientific Committees SCHER, SCCP and SCENIHR (2008)

The EU Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) have recently published a draft opinion on 'Use of the Threshold of Toxicological Concern (TTC) Approach for the Safety Assessment of Chemical Substances' (DG SANCO 2008).

The three Scientific Committees SCCP/SCHER/SCENIHR, in this section of this report referred to as the 'DG SANCO Expert Group', were requested to critically review the COLIPA Expert Group report on the use of the TTC concept in the safety evaluation of cosmetic products (Kroes et al. 2007, described in section 5.5.1) and the publicly available scientific literature on the concept of TTC and answer the following questions:

"1. Does the SCCP/SCHER/SCENIHR consider the TTC approach appropriate for the human health risk assessment of chemical substances?
2. In elaborating their opinion(s), and if the available information allows it, the SCCP/SCHER/SCENIHR are asked to address the following:

a) The various product categories including cosmetic products, consumer products, and others where a significant exposure of consumers to chemical substances is likely to occur in normal use situations.
b) The distinction between intentionally added ingredients and substances present in a particular product as inadvertent contaminants
c) Identification of classes of chemicals, exposure situations, toxicity end points for which the TTC concept may be appropriate and those for which it may not be
d) The quantity and type of data (exposure, toxicity, QSAR, statistics, etc) that will need to be available for a particular class of chemicals and/or exposure situation before the TTC concept can be applied in the risk assessment of chemicals
e) Additional research needed to strengthen the Threshold of Toxicological Concern approach and its usefulness for the human health risk assessment of chemical substances."

Initially, the draft opinion presents the history and development of the TTC concept, and describes the toxicological databases and use of the TTC concept in risk assessment; these parts in the draft opinion are covered by the previous sections in chapter 3 in this report. Then the current applications of the TTC concept are described followed by a presentation of potential applications of the TTC concept; these parts correspond to chapter 4 and 5, respectively, in this report. Finally, there is a discussion of potential applications and research needs.

The most important general views and recommendations from the draft opinion are presented below and, at the end of this section, the draft opinion is reproduced.

3.7.1 Use of the TTC concept in risk assessment: General aspects

Section 3.4 in the draft opinion presents some general considerations in relation to use of the TTC concept in risk assessment, section 3.7.1 in the draft opinion presents a discussion of the general aspects in relation to use of the TTC concept in risk assessment, and section 3.8 in the draft opinion presents the research needs as identified by the DG SANCO Expert Group.

The DG SANCO Expert Group clearly stated that the application of the TTC concept in risk assessment in any area requires a high level of confidence in the quality and completeness of the databases upon which the decision tree is based as well as the reliability of the exposure data for the intended use of the compound under study. It is the opinion of the Scientific Committees that in both of these areas further research is needed.

3.7.1.1 Toxicological databases

The DG SANCO Expert Group noted that the databases used to develop the TTC concept are exclusively based on systemic effects after oral administration of the chemicals: One is the carcinogenicity database containing 709 carcinogens (Cheeseman et al. 1999, described in section 3.3) and the other containing 613 chemicals is based on other systemic toxicological endpoints (Munro et al. 1996, described in section 3.2).
The DG SANCO Expert Group expressed the view that the carcinogenicity database, developed nearly 25 years ago (Gold et al. 1984) and expanded in 1999 (Cheeseman et al. 1999) should be reviewed with regards to the quality of the data included. They also found that it should be established to what extent the substances in the database can be considered to be carcinogenic according to the current guidelines for classification of carcinogens.
The DG SANCO Expert Group noted that the non-cancer toxicological endpoints database (Munro et al. 1996) is based upon data from the Registry of Toxic Effects of Chemical Substances (RTECS) and since this database is known to include data without a preliminary in depth quality check, the data used in the non-cancer database also require an in-depth quality control.

The DG SANCO Expert Group also expressed the view that an evaluation of more recent toxicity data is needed. They noted that, in case the TTC concept would acquire general use, the carcinogenic and non-cancer database should be continuously updated with newly available information on the substances they contain.

The DG SANCO Expert Group stated that the following aspects with regard to data entry need to be addressed:

- The databases must contain up to date and peer-reviewed data
- When new data are introduced, they need to be displayed under the same form as the existing data, meaning that they need to be the same type of result of the same type of test (e.g. NOAEL/NOEL/LOAEL/LOAEL from 28-day/90-day/chronic studies with rats/mice/dogs). Furthermore, correction factors (e.g. regarding allometry, study duration and study outcome) should be considered
- The database must contain a sufficient number of structure analogues to the compounds under study

The DG SANCO Expert Group mentioned that, in addition to the two above-mentioned databases, preliminary information has been presented on a database 'RepDose' containing 578 industrial chemicals based on both oral and inhalation exposure, described in section 5.1.4. The DG SANCO Expert Group noted that, for the oral route, the RepDose database gives lower values than the Munro database for all 3 Cramer classes and that the number of Cramer Class II chemicals is very small in both databases, about 20 chemicals (ca 4% of the total number of chemicals in the databases).
The DG SANCO Expert Group also noted that, for the RepDose database, all derived inhalation TTC values are lower than those for oral exposure which, according to the authors (of the publication presenting the RepDose database), might be due to the inclusion of local effects (e.g. irritation) in addition to systemic effects.

The DG SANCO Expert Group noted that there is a major overlap of NOELs and LOELs between Cramer Class I, II and III. Therefore, the DG SANCO Expert Group expressed the view that a better separation of the Cramer classes and consequently the TTC values based on them is needed. As the Cramer classification was developed in 1978 on theoretical considerations, the DG SANCO Expert Group found that it might be improved by analysis of outliers in the Classes and the incorporating recent experience on QSAR and modes of action into the decision tree.

For acceptance of the TTC concept for a specific area, the DG SANCO Expert Group considered it necessary to evaluate whether the chemical classes relevant for this area are covered by these databases. Furthermore, it was considered obvious that when there is no database containing certain groups of chemicals or certain endpoints the TTC concept can not be used for these chemicals or endpoints.

With reference to Kroes et al. (2004) and Barlow (2005), both described in section 3.4 in this report, the DG SANCO Expert Group noted that the following chemical groups should be excluded from the general TTC concept:

- Heavy metals and polyhalogenated dibenzo-p-dioxins, polyhalogenated dibenzofurans and polyhalogenated biphenyls, or any other compound known to accumulate in the body
- Endocrine disrupting chemicals, including steroids
- High molecular weight chemicals, such as polymers
- Organophosphates
- Proteins

Since the original data set was built for substances to be used in food contact materials the DG SANCO Expert Group noted that it is possible that, in other sectors, additional structural alerts could be identified which also need exclusion from the approach. They also noted that it is possible that for each sector, a specific decision tree will need to developed and applied, especially in view of the diverging exposure scenarios.

The DG SANCO Expert Group further emphasized that:

- no database is available that would allow application of the TTC concept to endpoints like allergic reactions, hypersensitivity, intolerance and local effects
- the TTC concept is not applicable to particulate matters including nano-materials since the knowledge is limited or to the endpoints pharmacological or microbiological effects since no database is available
- in order to extend the TTC concept to non-oral exposures, appropriate methodologies need to be developed to allow route-to-route extrapolation
- combined exposures in terms of exposure to multiple chemicals with the same mode of action should be given attention
- when using the TTC concept under REACH, no information on classification and labelling of a chemical or on its potency for a certain effect is provided

3.7.1.2 Exposure data

The DG SANCO Expert Group emphasized that exposure data are essential in any risk assessment procedure. In case they are lacking or are of insufficient scientific quality, worst case scenarios are regularly applied. As the TTC concept introduces an additional level of uncertainty in the hazard assessment by deriving the expected hazard, the Expert Group stressed that the need for sound exposure data becomes even more imminent.

In order to apply the TTC concept in risk assessment, the DG SANCO Expert Group noted that information on human exposure (consumers, workers, general population exposed via environment) is of crucial importance. Therefore, the importance to ensure that exposure estimates are as complete and accurate as possible, or that they are built on adequate conservatism to account for possible underestimates is clearly stressed by the DG SANCO Expert Group. They also noted that it is important to identify all exposure pathways to estimate the total exposure as humans are exposed to chemicals via ingestion, inhalation or dermal uptake. In particular, they stated that it is necessary to develop methodology to assess combined multi-route or multi-pathway exposures as such methodology is not yet available.

For application of the TTC concept and risk assessment of consumer products, the DG SANCO Expert Group found that generation of high quality exposure data is needed and substantial research in this area is required. This also includes research on systemic exposure after dermal and inhalation exposure. The DG SANCO Expert Group found that research is needed for consumer products in the following areas:

- use frequency and amount used
- duration of product contact
- concentration
- emission or leaching of a substance from the product to the skin or air
- and subsequently, absorption via the skin and/or the lungs or via oral route

3.7.2 Current applications of the TTC concept

Section 3.5 in the draft opinion addresses the applications of the TTC concept for food contact materials, flavouring substances, genotoxic impurities in pharmaceuticals, genotoxic constituents of herbal medicinal products / preparations, and industrial chemicals.

3.7.2.1 Food contact materials

The DG SANCO Expert Group noted that the US-FDA Threshold of Regulation (TR) for substances in food packaging was the first instance where a TTC-like approach was introduced by a regulatory body (described in section 3.1 and 4.1.1).
The DG SANCO Expert Group also noted that, in the EU the TTC concept is currently not used in the approval process of food contact materials, but that the European Food Safety Authority's (EFSA) Panel dealing with food contact materials applies a tiered approach to safety testing requirements and that this tiered approach has some similarities with the philosophy of the TTC concept (described in section 4.1.2).

3.7.2.2 Flavouring substances

The DG SANCO Expert Group noted that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has adopted the TTC concept in its evaluation of flavouring substances (described in section 4.2.1) and that the EFSA also uses the TTC concept for the assessment of flavouring substances in food (described in section 4.2.2), but that the TTC concept is used slightly differently by the EFSA and the JECFA.

The DG SANCO Expert Group concluded that the experience with the TTC concept for flavouring substances shows that the TTC concept can be used for a large number of chemical substances.

3.7.2.3 Genotoxic impurities in pharmaceuticals

The DG SANCO Expert Group noted that the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) has released a "Guideline on the Limits of Genotoxic Impurities", which recommends the application of a TTC value for defining acceptable limits of genotoxic impurities present in drug substances (described in section 4.4.1.1).
The DG SANCO Expert Group also noted that also the US-FDA's Center for Drug Evaluation and Research is considering the use of a TTC-based limit for regulation of genotoxic and carcinogenic impurities in drug substances.

The DG SANCO Expert Group concluded that regulatory experiences with the TTC concept since coming into force of the CHMP guideline in January 2007 show that this concept can be used as a pragmatic and very helpful tool for the regulation of genotoxic impurities in new drug substances.

3.7.2.4 Genotoxic constituents of herbal medicinal products / preparations

The DG SANCO Expert Group noted the EMA Committee on Herbal Medicinal Products/preparations (HMPC) has published a guideline on the assessment of genotoxicity of herbal medicinal products/preparations, which

allows using a TTC approach for the risk assessment of herbal preparations containing an identifiable genotoxic compound.
The DG SANCO Expert Group also noted that the TTC concept as currently applied is not validated for mixtures and preparations with often variable composition and for which a complete chemical characterisation is often not available. Furthermore, that plant extracts are not part of the databases used in the derivation of the TTC concept. And, with reference to EMA (EMEA 2008), that because of limited experience in the risk assessment of genotoxicity of herbal medicinal products a "lot of latitude in argumentation and justification has been allowed to the applicant" by the HMPC.

3.7.2.5 Industrial chemicals

The DG SANCO Expert Group noted that the REACH Regulation provides the possibility to waive testing of a substance based on the scenarios developed in the exposure assessment, but that there is no reference to any thresholds (described in section 5.1.1). In the guidance document for the implementation of REACH (REACH TGD) (ECHA 2008a) information on criteria for waiving certain studies is provided, including for the use of the TTC concept which is considered a helpful tool under REACH, especially in the case of waiving certain studies (described in section 5.1.1).
The DG SANCO Expert Group also noted that when using the TTC concept under REACH no information on classification and labelling of a chemical or on its potency for a certain effect is provided.

It should be noted that, in the draft opinion, the section on industrial chemicals is placed under the subtitle 'Current applications'. However, the TTC concept has not been implemented in the REACH Regulation, but only addressed in an Annex to the REACH TGD (ECHA 2008a). Therefore, the section on industrial chemicals is placed in Chapter 5 'Discussions on potential applications of the TTC concept' in this report.

3.7.3 Potential applications of the TTC concept

Section 3.6 in the draft opinion addresses the potential applications of the TTC concept for:

- food additives
- residues from veterinary medicinal products
- drinking water contaminants and materials intended for contact with drinking water
- cosmetic products
- allergic contact dermatitis
- genotoxic impurities in veterinary medicinal products
- medical devices
- consumer products including household care products, and
- air pollutants.

3.7.3.1 Food additives

The DG SANCO Expert Group noted that, according to the regulation only additives that have undergone a full toxicological evaluation are authorized and therefore, the TTC concept is not considered relevant so far in the risk assessment of the food additives.

The DG SANCO Expert Group also noted that an EFSA TTC Working Group was adopted at the 31st plenary meeting of the EFSA Scientific Committee (described in section 5.2).

The DG SANCO Expert Group concluded that the TTC concept may possibly be used if an unsuspected chemical or impurity be detected in a food additive. And that in future, other areas of risk assessment may be included.

3.7.3.2 Residues from veterinary medicinal products

The DG SANCO Expert Group noted that, in the Commission Regulation for residues of veterinary medicinal product in food commodities, a TTC-like consideration have been taken into account on a case by case basis for certain substances with a relatively low risk profile, but the TTC concept, as a scientific concept, has not been applied yet.
The DG SANCO Expert Group also noted that the Scientific Committee of Veterinary Medicinal Products (CVMP) has already identified some scenarios where the TTC concept may be appropriate (described in section 5.3).

The DG SANCO Expert Group concluded that the TTC concept is currently not used in the assessment of consumer safety of residues of veterinary medicinal products in food. And that the TTC concept might offer an appropriate option in the assessment of substances that have no ADI/MRL and certain impurities/trace level residue concentrations. As well as that it should be noted that endpoints like pharmacological or microbiological effects are not addressed in the currently available databases.

3.7.3.3 Drinking water contaminants and materials intended for contact with drinking water

The DG SANCO Expert Group noted that, for pesticide metabolites in groundwater, the former EC Scientific Committee on Plants proposed a TTC approach in its opinion regarding the draft guidance document on relevant metabolites (described in section 4.3).
The DG SANCO Expert Group also noted that the EC Scientific Committee on Plants has concluded that the TTC concept is a valid tool to be used in the process of risk assessment of metabolites and, under the proposed conditions of use, can provide an adequate margin of protection and a reliable evaluation of the need for a more complete risk assessment of metabolites of plant protection products.

3.7.3.4 Cosmetic products

In section 3.6.2 in the draft opinion, a detailed description of the possible use of the TTC concept for the safety evaluation of cosmetic ingredients based on the workshop organized by COLIPA and reported by Kroes et al. (2007) is presented. This section in the draft opinion covers, more or less the description presented in section 5.5.1 in this report.

Section 3.7.2 in the draft opinion presents a detailed discussion of the potential applications for cosmetics as the main term of reference to the DG SANCO Expert Group was to critically review the COLIPA Expert Group report on the use of the TTC concept in the safety evaluation of cosmetic products (Kroes et al. 2007). The following questions were analysed and discussed; the most important aspects are presented below:

1. To what extent can the available databases be used in the case of cosmetic ingredients considering structural similarities/dissimilarities between cosmetic ingredients and substances in the existing databases?
2. What are the differences in metabolism between dermal and per oral routes of application?
3. How to address skin contact allergies and other topical effects?
4. How should exposure be assessed?
5. Should intentionally added or formed ingredients in cosmetic products and inadvertent contaminants and impurities be considered differently?

Ad 1.
In a preliminary search in the CosIng database (containing about 15,000 cosmetic ingredients) using CAS numbers, 251 chemicals that are also present in the database of Munro et al. (1996, described in section 3.2) were identified. Of these, 96 are banned from the use in cosmetic products and of the remaining 155 chemicals, 101, 17 and 37 are in Cramer Classes I, II and III, respectively. These 155 chemicals can be grouped in approximately 25 chemical categories based on chemical structure.
In a separate analysis, the 250 chemicals that have been evaluated by SCCNFP/SCCP (1997-2007) were compared with the Munro data base. Only 19 of the 250 chemicals (7.6%) were found in the Munro database. Of these, 11, 1 and 7 are in Cramer Classes I, II and III, respectively.
Of the 19 chemicals, NO(A)ELs are available for 13 substances. For these, a comparison was made between acceptable doses derived from the toxicological data (NO(A)EL) and exposure limits according to the TTC concept. For 4 of these ingredients, the acceptable levels according to the TTC concept are higher than the maximum doses determined by conventional risk assessments.
Based on these analyses, the DG SANCO Expert Group concluded that a revised and adequate toxicity database of relevance to cosmetic ingredients of various chemical categories, within all three Cramer Classes, and with reliable NO(A)EL will be required before the safety evaluation of ingredients in cosmetic products employing the TTC concept can be conducted with confidence.

Ad 2.
The major enzymes found in the liver may also be present in the skin, but at lower activity levels compared to other tissues. There are examples that only small percentages of absorbed substances are metabolized. On the other hand, in some cases complete biotransformation during dermal absorption was observed. Detoxification capacity (phase II enzymes) may be even more pronounced in the skin. Oxidative bioactivation of prohaptens to haptens in the skin is considered a hazard of xenobiotics applied topically.
As the databases used to develop the TTC concept comprise experiments with oral administration of the chemicals, the DG SANCO Expert Group found that, in order to extend the TTC concept to non-oral exposures, appropriate methodologies need to be developed to allow route-to-route extrapolation taking into account the potential for skin metabolism and/or biotranformation/bioactivation.

Ad 3.
Kroes et al. (2007) recognized that the TTC concept cannot at present be used to evaluate local effects (described in section 5.5.1). Although the

authors considered application to such effects potentially possible, they found that the databases on local effects, such as sensitization or irritation, and on substances producing these effects, are currently too limited to be used as a basis for the derivation of valid TTC values for local endpoints. The DG SANCO Expert Group agreed with this and thus, concluded that the TTC concept is not applicable for the safety evaluation of allergic contact dermatitis and of other local effects at the site of application (e.g. contact allergies, irritation, phototoxicity), which are important endpoints for the safety assessment of cosmetic ingredients.
The DG SANCO Expert Group noted that the proposal to use the TTC concept for dermal sensitisation (Safford 2008, described in section 5.10.1) based on the dermal sensitisation QRA (Quantitative Risk Assessment) method may, after refinement and validation, in the future be applicable for risk assessment of new substances to suggest a safe level of exposure prior to incorporation into products.

Ad 4.
The DG SANCO Expert Group noted that, concerning exposure assessment of chemicals used in cosmetics, Kroes et al. (2007) made a number of recommendations concerning the use of default adjustment factors for percutaneous absorption, rinse off cosmetic products, and the intermittent use of cosmetic products. Some critical points of the proposal of default adjustment factors for percutaneous absorption are presented in the draft opinion (section 3.7.2.3). Although the use of an adjustment factor for percutaneous absorption in the absence of experimental data is promising according to the DG SANCO Expert Group, they considered that this proposal is far from being sufficiently developed and should be further validated based on a broad systematic comparison of predicted and experimentally obtained percutaneous absorption values.

Ad 5.
Based on the above considerations and limitations concerning the available data bases, the skin to oral route extrapolation, and the exposure assessments the DG SANCO Expert Group concluded that the TTC concept as proposed by Kroes et al. (2007) is at present in general not applicable for risk assessment of intentionally added or formed ingredients present in cosmetic products. The same conclusion can be reached for impurities in cosmetic ingredients. In the future with validated extended databases and percutaneous absorption default factors and adequate knowledge on skin-oral route metabolism and biotransformation differences, the application for cosmetic ingredients and impurities could be further considered.

3.7.3.5 Allergic contact dermatitis

The DG SANCO Expert Group noted that, in the present form, the TTC concept refers to systemic toxicity, in which allergic contact dermatitis is not considered as an endpoint. This is further addressed in the preceding section.
The DG SANCO Expert Group also noted that a proposal was recently published to use the TTC concept for dermal sensitisation as well (described in section 5.10.1).

3.7.3.6 Genotoxic impurities in veterinary medicinal products

The DG SANCO Expert Group noted that there is ongoing discussion to introduce a limit for genotoxic impurities in veterinary medicinal products

similar as has been adopted for human medicinal products (described in section 4.4.1.1), not only in relation to animal safety but also as reference point for user safety and possibly consumer safety evaluations, but that these discussions are at a preliminary stage.

3.7.3.7 Medical devices

The DG SANCO Expert Group noted that, currently, the TTC concept is not used for evaluation of medical devices, but that the TTC concept is attractive for the use in the medical device area for chemical residues of production processes and that the TTC concept has recently become a subject of discussion for possible application in the medical device area.

3.7.3.8 Consumer products including household care products

The DG SANCO Expert Group noted that, currently, for chemicals used in consumer products the TTC concept is not used, but that this might change in the future according to a possible broader use of the TTC concept within the REACH process.
The DG SANCO Expert Group also noted that the applicability of the TTC database to ingredients in personal and household care products has been evaluated recently by Blackburn et al. (2005, described in section 5.6).

3.7.3.9 Air pollutants

The DG SANCO Expert Group noted that Drew and Frangos (2007) have developed a "Concentration of No Toxicological Concern" (CoNTC) as a screening tool for air pollutants, described in section 5.9.1.

3.7.4 The opinion

The opinion of the DG SANCO Expert Group, section 4 in the draft opinion, is reproduced below:

"The Threshold of Toxicological Concern (TTC) approach is a risk assessment tool that is at present used to evaluate safety of chemicals that occur at low levels. Currently it has been used for food contact materials, flavouring agents and genotoxic contaminants in pharmaceuticals. The approach has been suggested for a number of other application areas.

The TTC concept is based on the principle of establishing a generic human exposure threshold value for chemicals, below which there is a low probability of systemic adverse effects to human health. The concept is based on extrapolation of toxicity data from an available database to a chemical compound for which the chemical structure is known, but no or limited toxicity data is available. From a scientific point of view, in principle, the TTC approach is applicable to any substance be it an intentionally added ingredient or a substance present in a particular product as inadvertent contaminant or impurity.

The principle of the TTC approach in itself is scientifically acceptable. However, the application of this principle in terms of risk assessment for safety evaluation of a chemical is dependent on the quality, quantity, and relevance of the underlying toxicity database, and a reliable estimation of the exposure to the chemical in the respective field of application.

One carcinogenicity database (Cheeseman et al. 1999) containing 709 carcinogens and one (Munro et al. 1996) based on other toxicological endpoints containing 613 chemicals have been used for derivation of the TTC values. Both are exclusively based on systemic effects after oral exposure. Several classes of chemicals have been identified, for which the TTC concept can not be applied. Also for certain endpoints, like allergic reactions, intolerance, local effects and pharmacological effects, the approach can presently not be applied. Additional limitations exist with regard to extrapolation to other exposure routes (inhalation and dermal). Recently published preliminary data on the RepDose database suggest that there is some doubt about the classification system by Cramer and that refinements are needed.

Appropriate exposure assessment is essential for TTC. In the case of genotoxic contaminants in pharmaceuticals, and food flavourings, where TTC is already in use, the available information has been considered adequate. Limited knowledge exists in other areas, e.g. for consumer products, where a large diversity of products exists and complex exposure scenarios have to be considered including multiple exposure routes. In this area, the uncertainties are higher and methodology is less developed. Significant exposure is likely for products that are frequently used. This may involve oral exposure or skin contact or exposure via inhalation by using e.g. cleaning products, cosmetics or toys. For many of these product categories, however, exposure data are limited or lacking.

In relation to cosmetic ingredients, the current database is considered inadequate. Therefore, the TTC approach is at present in general not applicable for intentionally added or formed ingredients present in cosmetic products. The same conclusion can be reached for impurities in cosmetic ingredients. In the future with validated extended databases and more experience, the application for chemicals in cosmetics and possibly other consumer products could be further considered.

Further research is needed in the development and validation of the current toxicity databases particularly in the areas where an insufficient number of representative chemicals is included. In addition, the methodology for assessing systemic exposure needs to be improved and appropriate data on exposure need to be generated for the various exposure scenarios."

4 Current applications of the TTC concept

The TTC concept is already being applied in different regulatory settings internationally, notably in the risk assessment of food contact materials, in the evaluation of flavouring substances in food, and in the evaluation of genotoxic constituents in pharmaceuticals for humans and in herbal medicinal products and preparations. This Chapter outlines these actual applications of the TTC concept.

4.1 Food contact materials

4.1.1 U.S. Food and Drug Administration (US-FDA)

The U.S. Food and Drug Administration (US-FDA) has used the general TTC concept for establishing a Threshold of Regulation (TR) for food contact materials when the overall dietary concentration of an identified migrant of known chemical structure is below 0.5 µg/kg food, which equals an intake of 1.5 µg/person/day, assuming a total daily intake of food and drink of 3 kg (1.5 kg of solid food and 1.5 kg of liquid food) for an adult person with a body weight of 60 kg. In this case no toxicity testing is required, although CAS number, chemical structure, and a toxicity profile based on available data is requested. Above this threshold, the degree of required testing increases as the estimated exposure increases (Table 6).

Table 6. US-FDA tiered approach for toxicity testing of chemicals migrating from food contact materials. (After US-FDA 2002).

Dietary concentration of migrant	Toxicity tests required
<0.5 µg/kg	No testing required, although a toxicity profile based on available data is requested.
>0.5 µg/kg - <50 µg/kg	Only genotoxicity tests (e.g. *in vitro* bacterial mutagenicity tests and an *in vitro* test with cytogenetic evaluation of chromosomal damage using mammalian cells or (preferably) an *in vitro* mouse lymphoma $tk^{+/-}$ assay) are required.
>50 µg/kg - <1 mg/kg	Additional genotoxicity tests (e.g. *in vivo* rodent assay for chromosomal damage) and two sub-chronic oral toxicity test are required (one in a rodent and one in a non-rodent species
>1 mg/kg	Complete toxicology testing may be required, as specified for direct food additives

The Threshold of Regulation (TR) is assumed to protect against all types of toxicity, including carcinogenicity. However, substances containing structural alerts indicative of potential carcinogenicity, and substances with evidence for carcinogenicity in animals or humans should not be exempted from full testing. A useful list of such structural alerts indicating carcinogenicity is given in Bailey et al. (2005). Complete details of the criteria for Threshold of Regulation exemption are given in Title 21 of the U.S. Code of Federal Regulations, section 170.39 (21 CFR 170.39).

Experience with the use of the Threshold of Regulation principle in the U.S.A. indicates that it is practical and cost saving (Barlow 2005).

4.1.2 The EU Commission (EC) and the European Food Safety Authority (EFSA)

In EU, food contact materials are regulated by the Framework Regulation (EC) 1935/2004, which sets up general requirements for all food contact materials, and by specific directives which cover single groups of materials and articles listed in the Framework Regulation, and directives on individual substances or groups of substances used in manufacture of materials and articles intended for food contact (EC 2006a). The legislation on plastics (Commission Directive 2002/72/EC) is the most developed and provisional lists of monomers and additives notified to the European Commission for use in the manufacture of plastics or coatings intended to come into contact with foodstuffs can be found in the European Commission Synoptic Document (EC 2005).

Two types of limits have been established for substances migrating from plastic materials:

- an Overall Migration Limit of 60 mg substance/kg food or food simulant, that applies to the sum of all substances that can migrate from food contact plastics to foods, and

- Specific Migration Limits (SML) or restrictions (Qm) are established by the European Commission. They apply to individual authorised substances and are derived on the basis of the toxicological evaluation of that substance performed by EFSA.

The restrictions for a given substance can thus be established in two different ways:

- For compounds with adequate toxicological data to set an acceptable daily intake (ADI) or tolerable daily intake (TDI) the SML should take care that the ADI/TDI of the substance is not exceeded by a person weighing 60 kg who daily throughout life eats 1 kg food packed in plastics material containing the substance.
- For compounds with a reduced toxicological data set, the limits are set according to Table 7. For instance, when only genotoxicity data are available the migration must not exceed 0.05 mg/kg food.

A plastic material is considered acceptable for packaging of food if the migration into the food or appropriate simulants (water, 3% acetic acid, 10% ethanol, or olive oil or other fat simulants) is below the SML or Qm.

A threshold of regulation approach is adopted in the amendment of Directive 2002/72/EC for substances in layers of multi-layer plastic material where the layer is an outer layer behind a functional barrier and not in direct contact with foods. In such cases the migration of the substances into food or food simulants should not exceed 0.01 mg/kg. This value is regarded as a detection limit. The chemicals migrating must not be mutagenic nor carcinogenic.

EFSA has not formally used the TTC concept in the assessment of food contact materials. However, in the evaluation of migration of food contact materials thresholds are used to decide the amount of toxicity data needed to be supplied by the petitioner to the EFSA (EFSA 2005). As a general

principle, the greater the exposure through migration, the more toxicological information will be required (see Table 7).

Table 7. EFSA tiered approach for toxicity testing of chemicals migrating from food contact materials (EFSA 2006).

Degree of migration	Toxicity tests required
<0.05 mg/kg food	Three *in vitro* mutagenicity tests: • A test for gene mutations in bacteria • A test for gene mutations in mammalian cells • A test for chromosomal aberrations in mammalian cells
0.05-5 mg/kg food	• 3 *in vitro* mutagenicity tests as above • A 90-day oral toxicity study • Data to demonstrate the absence of potential to accumulate in man
5-60 mg/kg food	• 3 *in vitro* mutagenicity tests as above • 90-day oral toxicity studies, normally in two species • Studies on absorption, distribution, metabolism and excretion • Studies on reproduction in one species, and developmental toxicity, normally in two species • Studies on long-term toxicity / carcinogenicity, normally in two species

4.2 Flavouring substances

The two main types of flavouring ingredients used in food are the chemically defined flavourings and the naturally occurring flavour mixtures isolated primarily from plants (Smith et al. 2005). More than 2800 different chemically defined flavouring substances are claimed by industry to be currently added to foods and beverages in Europe or the U.S.A. In addition, about 400 natural flavour complexes are in use. The chemically defined flavourings are volatile or semi-volatile organic chemicals, and the majority has simple, well-characterized structures with a single functional group and low molecular weight (<300 g/mol) (Munro et al. 1999). The vast majority of chemically defined flavourings exists naturally in foods or is formed during preparation of foods via heating and mixing. A number of chemically defined flavourings that are produced by synthesis have not been found in nature, but are structurally related to naturally occurring flavourings. Regardless of their origin, essentially all chemically defined flavourings belong to approximately 40 well-defined structural chemical groups. Each flavour ingredients can therefore be evaluated individually and within the context of its chemical group. If the structure of a substance under evaluation can be assigned to a well-defined chemical group for which safety data exists, the substance in question can be evaluated even if little or no toxicological data exists on that particular substance.

4.2.1 FAO/WHO Joint Expert Committee on Food Additives (JECFA)

Up till 1995, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) had only evaluated about 70 of the approximately 3000 flavouring agents used in foods (Larsen 2006). In order to speed up the evaluation of flavouring substances, JECFA adopted a Procedure for the Safety Evaluation of flavouring Agents. The procedure was first discussed in 1995 at the 44th meeting of JECFA based on a paper prepared by Dr. I. C. Munro (WHO 1995, IPCS 1996, Munro et al. 1996) which introduced the structure-based, tiered TTC approach (described in section 3.2). The procedure was endorsed with modifications in 1996 at the 46th meeting of JECFA (WHO 1997) and used for the evaluation of a number of flavouring substances being simple esters. The procedure was further modified in 1997 at the 49th meeting of

JECFA to include the acceptance of a general threshold of toxicological concern of 1.5 µg/person/day (WHO 1999, IPCS 1998, Munro et al. 1999).

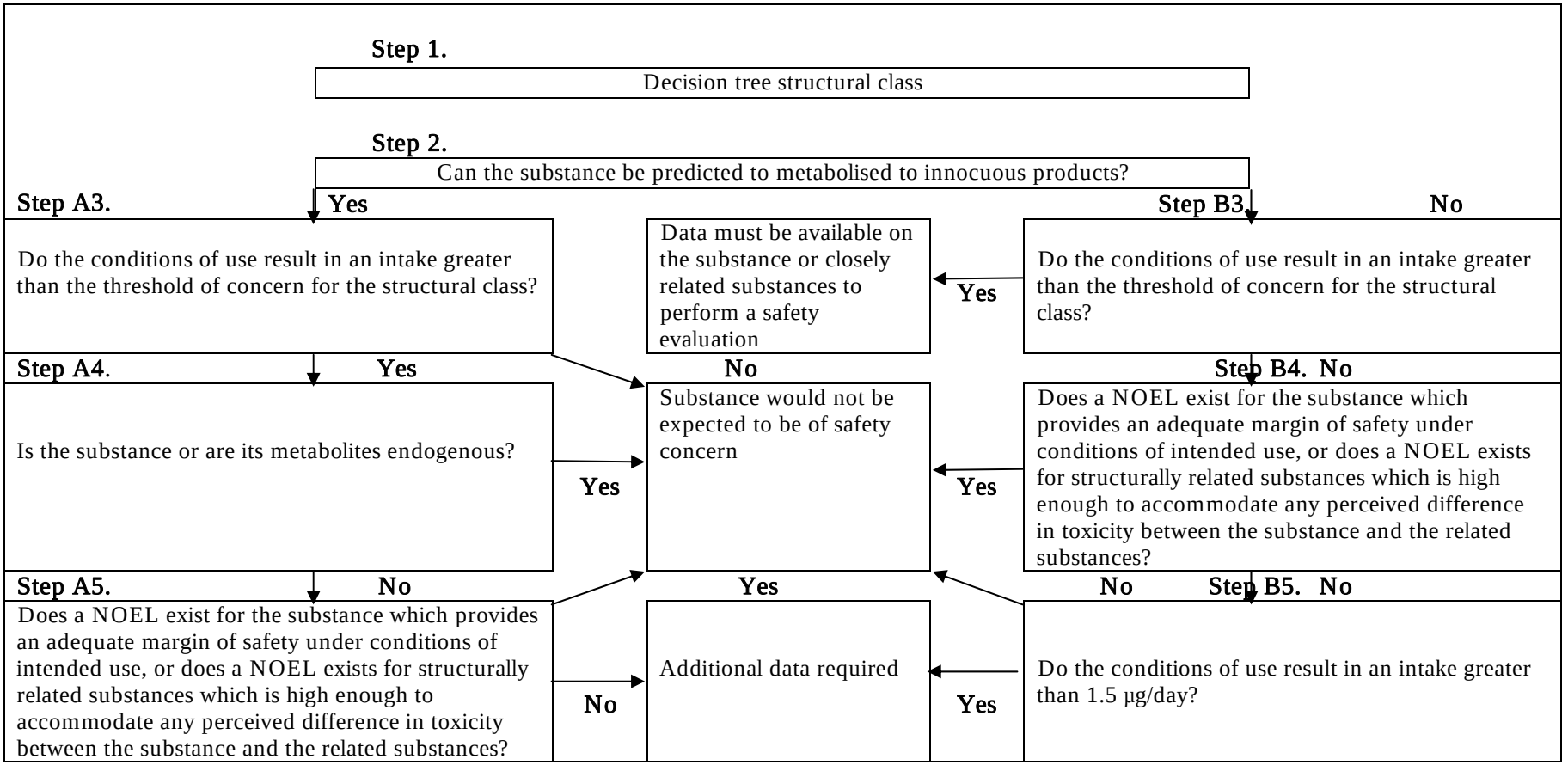
The procedure takes into account available information on structure-activity relationships, metabolism, intake and toxicity data on groups of structurally related flavouring substances. The procedure for the safety evaluation of flavouring agents proceeds through a number of steps in which several questions have to be answered (Figure 4). First, the substances are classified into Class I, II, or III according to their chemical structures (Cramer et al. 1978). As already mentioned in section 3.2 structural Class I embraces substances with presumptive low toxicity, structural Class II are substances with presumptive medium toxicity, while structural Class III might contain substances with significant toxicity. The TTC values for each structural class are 1800, 540 and 90 µg/person/day, for Class I, II and III, respectively (Munro et al. 1996, 1999), see section 3.2. Thereafter, it is considered whether the substances can be metabolised to innocuous products or not. If the answer to this question is "yes", the evaluation proceeds along the A-side of the scheme, and the next step is to examine whether the estimated intake of the substance is greater than the TTC value for its structural class, calculated by the Maximised Survey-derived Daily Intake (MSDI) method (see below). If the intake is below the respective TTC value, the flavouring would not be expected to be of safety concern. If the intake is above the TTC value, but the flavouring or its metabolites are endogenous, the same conclusion is reached. If they are not endogenous, toxicity data has to be taken into account.

The evaluation of flavourings that cannot be predicted to be metabolised to innocuous products proceeds via the B-side of the scheme. For flavourings with an estimated intake above the respective TTC value, more data are required on the compound or closely related substances to perform a safety evaluation. For flavourings with an intake below the respective TTC value, toxicity data are also required in order to derive a NOAEL for the substance or a closely related substance. From the NOAEL value it is determined whether there is an adequate margin of safety under the condition of intended use. If the margin is high enough the substance will not be considered to be of a safety concern. If the margin is low, additional data on the substance are required.

Using this procedure JECFA has now evaluated more than 50 groups of flavourings containing a total of more than 2000 flavouring substances.

The intake estimation is an important step in the safety evaluation of flavouring substances by the TTC concept. In the absence of other suitable and better methods, JECFA has until now used the Maximised Survey-Derived Daily Intake (MSDI) method (also called the 10 x ***per capita*** method), which is derived from the annual production volume of flavourings as reported for USA (data from the U.S. National Academy of Sciences/National Research Council) and Europe (data from International Organization of the Flavour Industry (IOFI)), respectively.

Figure 4: Procedure for Safety Evaluation of Chemically defined Flavouring Substances



The estimates were based on the assumption that the surveys accounted for only 60% of the production and that the entire amount produced was consumed by only 10% of the population ("eaters only"):

$$\text{Intake (µg/person/day)} = \frac{\text{annual volume of production (kg)} \times 10^{9}\ \text{(µg/kg)}}{\text{population of consumers} \times 0.6 \times 365\ \text{days}}$$

Using the MSDI method for intake estimations has several limitations. The MSDI data for Europe were for a number of years derived from surveys on annual production volumes from 1995 and the population of consumers was assumed to be 32×10^{6} in the Europe (EU) in 1995. However, new production figures for Europe were provided in 2005. The MSDI model does also not take into account the consumption pattern of subgroups in the population. It does neither consider geographic variations in the use of the flavourings, nor the fact that a specific flavouring substance can be used only in one or very few food categories. Consequently, since 2007 JECFA has used an alternative method for the dietary exposure estimation called the "Single Portion Exposure Technique" (SPET) which provides a dietary exposure estimate based on use levels recommended by the industry and aims to represent the chronic dietary exposure for a regular consumer who consumes daily a specific food product containing the flavouring agent of interest. The SPET identifies the single food category containing the flavouring agent of interest that is likely to contribute the highest dietary exposure based on a "standard portion" size.

4.2.2 European Food Safety Authority (EFSA)

The procedure adopted by JECFA in 1997 (WHO 1997, 1999) was evaluated by the EC Scientific Committee for Food (SCF) in 1999, and adopted for use in EU with some modification for evaluation of flavouring substances (SCF 1999). In 2003, the Scientific Panel on Food Additives, Flavouring Agents, Production Aids and Food Contact Materials (AFC Panel) under the European Food Safety Authority (EFSA) took over the evaluations of flavouring substances in food after the SCF and also used this approach for the assessment of flavouring substances in food (Larsen 2006). In 2008 this task fell into the remit of the Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing aids (CEF Panel).

Like JECFA, the EFSA Panel evaluates flavouring substances in numbered groups of structurally related compounds and the opinions are termed Flavouring Group Evaluations (FGEs). Up to almost 50 FGEs have been performed so far.

The CEF Panel does not use the general TTC value of 1.5 µg/person/day. The rejection of the TTC value of 1.5 µg/person/day originates from the SCF evaluation in 1996 of the use of the threshold of toxicological concern concept in the evaluation of food contact material (SCF 1996) and the SCF opinion in 1999 of the programme for evaluation of flavouring substances (SCF 1999). The SCF in 1996 was of the opinion that "***before any firm conclusion could be reached on a dietary limit value for a threshold of no toxicological concern for non-genotoxic endpoints, it would be necessary to conduct an up-to-date review of existing data covering important endpoints of concern which may give rise to effects at low doses, such as neurotoxic, immunotoxic, endocrinologic and developmentally***

toxic events". In addition, the SCF considered that "***the remaining risk from exposure to chemicals at very low doses is most likely to be heritable and carcinogenic risks from genotoxic chemicals. Present scientific knowledge does not allow a definitive conclusion as to whether or not a true threshold exists for genotoxic carcinogens***".

In its 1999 opinion the SCF noted that the potential genotoxicity of the substances is not explicitly addressed in the JECFA procedure, although a clearly genotoxic and carcinogenic compound would not be taken through the procedure by JECFA. According to the SCF (1999), flavouring substances should also be examined for structural alerts for genotoxicity and if structural alerts or test results indicate that a substance is likely to be genotoxic, it should not be carried through the procedure. EFSA also does not accept to evaluate a (non-genotoxic) flavouring substance on the sole basis that the estimated intake of this substance is lower than the TTC value of 1.5 μg/person/day, as is used by JECFA.

The EFSA Panels have also performed additional intake estimates (than the MSDI) using a modified theoretically anticipated maximum daily intake (M-TAMDI) approach. Although both are used in the opinions adopted by EFSA, the final evaluations are based on the MSDI method. However, if a calculated M-TAMDI for a flavouring substance exceeds the relevant threshold for its structural class, more reliable exposure data are requested by which the substance will be re-evaluated (EFSA 2004).

The TAMDI-approach calculates intakes on the basis of standard portions and "upper" use levels (SCF 1995) for beverages and foods in general, with exceptional use levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level. It sometimes overestimates the intake by orders of magnitude. Because EFSA receive information of both "normal" and upper use level from the industry, EFSA modified the TAMDI-approach by basing the intake calculations on the normal rather than the upper use levels of the flavouring substances. This modified approach (M-TAMDI) is less conservative (e.g., it may underestimate the intake by consumers being loyal to products flavoured at the maximum use levels reported) (EC 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA 2004).

For the EFSA evaluations, the draft FGE opinions are prepared by the FLAVIS Working Group, being hosted by the DTU National Food Institute, Technical University of Denmark. They are thereafter evaluated by two Flavourings Working Groups under the CEF Panel and then passed on to the panel for final adoption. The end goal is to achieve an EU positive list of chemically defined flavouring substances. By July 2010 the database of flavouring substances contained 2803 substances.

The new Regulation (EC) No 1334/2008 of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties (EC 2008a) amending Council Regulation (EEC) no 1601/91 (EEC 1991), Regulations (EC) No 2232/96 (EC 1996) and (EC) No 110/2008 (EC 2008b) and Directive 2000/13/EC (EC 2000) was adopted on 16 December 2008. The Regulations entered into force on 20 January 2009.

The new regulation applies to flavourings which are used or intended to be used in or on foods, food ingredients with flavourings properties, food containing flavourings and/or food ingredients with flavouring properties and source materials for flavourings and/or source materials for food ingredients with flavouring properties. A guidance document on the data requirement for new flavouring substances has been issued by the CEF Panel (EFSA 2010). The initial approach in the assessment is still to consider whether a new compound, based on its chemical structure, falls into one of the existing FGEs. The guidance document also describes a new method to estimate the dietary exposure for adults and children. The new method is an adaptation of the TAMDI method called the "Added Portions Exposure Technique" (APET). The APET is more conservative than the SPET since it retains the assumption used under the TAMDI, that the consumer will daily consume a fixed amount of both flavoured solid foods and flavoured beverages.

4.2.3 Flavor and Extract Manufacturers Association (FEMA)

Since the development of the TTC concept in 1995, an Expert Panel under the Flavor and Extract Manufacturers Association (FEMA) for the safety evaluations of natural flavour complexes (NFC), such as essential oils, has adopted the TTC concept according to the chemical classes and thresholds described above for JECFA (Smith et al. 2005). They state that the TTC concept provides an efficient method to organize and prioritize the significant amount of data on the relatively large number of chemical constituents and chemical groups in a NFC, and can be used to evaluate the small amounts of unidentified substances in such a complex mixture. However, the TTC concept has so far not been introduced by JECFA or EFSA for the assessment of natural flavouring complexes.

4.3 Pesticide metabolites in groundwater

The TTC concept has been proposed in the EU in the assessment of certain metabolites of active substances of plant protection products in groundwater. A guidance document on the assessment of the relevance of metabolites of pesticides in groundwater has set criteria, based on the potential exposure and toxicological considerations, for the conditions in which a metabolite is to be considered relevant, and therefore its concentration in drinking water must not exceed 0.1 µg/L (DG SANCO 2003).

In practice, the guidance document (DG SANCO 2003) has been used for the assessment of the relevance of metabolites of pesticides in groundwater since it was taken ad notam in the Standing Committee on the Food Chain and Animal Health (personal communication 2010).

For metabolites considered to be not relevant, a TTC approach should be followed. For substances of unknown structure the EC Scientific Committee on Plants has proposed a TTC value of 1.5 µg/person/day (0.02 µg/kg bw/day for an adult person with a body weight of 70 kg. Assuming a consumption of 2 litres of water per day, such an acceptable exposure level relates to an acceptable upper limit for the concentration of the metabolite of 0.75 µg/L. This threshold is only considered acceptable if the metabolite in question (DG SANCO 2003):

- Does not exceed 0.75 µg/L (or a lower level if consumers are exposed also via other routes,

- Has a lower biological activity than the parent,
- Is not genotoxic,
- Is not classified as toxic, reprotoxic or carcinogenic.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have noted The EC Scientific Committee on Plants has concluded that the TTC concept is a valid tool to be used in the process of risk assessment of metabolites and, under the proposed conditions of use, can provide an adequate margin of protection and a reliable evaluation of the need for a more complete risk assessment of metabolites of plant protection products.

4.4 Pharmaceuticals for human use

4.4.1 Genotoxic impurities in pharmaceuticals

4.4.1.1 EMA/CHMP "Guideline on the Limits of Genotoxic Impurities" (EMEA 2006)

Regulations on how to control impurities in a drug substance are addressed in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Quality Guideline Q3A (ICH 2002). This document does not specifically provide guidance for impurities with a genotoxic potential.

The European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) has evaluated the use of the TTC concept in the risk assessment of genotoxic impurities in pharmaceuticals for human use. EMA considered the need for a pragmatic approach for toxicological assessment of genotoxic substances without sufficient evidence for a threshold-related mechanism, realizing that a complete elimination of such impurities from the drug substances is often unachievable.

In 2006, EMA/CHMP released a "Guideline on the Limits of Genotoxic Impurities" which came into effect on 1 January 2007 (EMEA 2006). More recently a Question and Answer (Q&A) document was published at the EMA website (EMEA 2007) which is intended to serve as a supplement of the guideline and addresses several aspects in relation to the practical implementation of the guideline's recommendations.

For genotoxic compounds without sufficient evidence for a threshold-related mechanism, the Guideline proposes the application of a TTC concept to determine acceptable impurity levels with reference to the paper of Kroes et al. (2004, described in section 3.4) where a TTC value of 0.15 µg/day is proposed for those substances with structural alerts for genotoxicity corresponding to a 10^{-6} lifetime risk of cancer. However, for application of a TTC concept in the assessment of acceptable limits of genotoxic impurities in drug substances, a value of 1.5 µg/day (corresponding to a 10^{-5} lifetime risk of cancer) was recommended since it was considered that benefits of pharmaceuticals would justify a lifetime risk of cancer of 10^{-5}.
The concentration limits of the genotoxic impurity level in a drug substance derived from the TTC value can then be calculated based on the expected daily dose to the patient.

In agreement with the papers of Kroes et al. (2004, described in section 3.4) and Cheeseman et al. (1999, described in section 3.3), some structural groups of high potency genotoxic carcinogens such as aflatoxin-like, azoxy-, and N-nitroso-compounds are excluded from the TTC concept. Risk assessment of genotoxic impurities belonging to such groups would require compound-specific toxicity data. It is also noted that the TTC concept should not be applied to carcinogens where adequate toxicity data (long-term studies) are available and allow for a compound-specific risk assessment.

The Guideline also stresses that, dependent on aspects of the clinical use of a drug product there may also be circumstances to accept higher limits than 1.5 µg/day, e.g., for short-term exposure, for treatment of a life-threatening condition, when life expectancy is less than 5 years, or when the impurity is a known substance and human exposure will be much greater from other sources, e.g., food. Genotoxic impurities that are also significant metabolite may be assessed based on the acceptability of the metabolites.

The Guideline focuses on orally applied drugs when recommending the TTC as acceptable limit and does not provide any specific recommendations for other routes of drug administration.

The Guideline includes a decision tree to assess the acceptability of genotoxic impurities.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have noted that this decision tree suggests applying a policy of controlling levels to “as low as reasonably practicable” (ALARP principle) implying that every effort should be made to prevent the formation of such impurities during synthesis and, if not possible, to reduce them through technical efforts, e.g. purification steps. According to this decision tree approach the ALARP principles precede the recommended application of a TTC concept and it seems that ALARP should be applied even in cases where the concentration of a genotoxic impurity does not exceed the TTC value. This issue has been clarified in the Q&A document which clearly states that if the level of a mutagenic impurity is below the threshold of toxicological concern (equivalent to a clinical dose < 1.5 µg/day) it is not necessary to apply ALARP considerations (EMEA 2007), i.e. a genotoxic impurity at the TTC value would be acceptable even if its formation could be simply avoided by using a known and established alternative route of synthesis.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have concluded that regulatory experiences with the TTC concept since coming into force of the EMA/CHMP Guideline in January 2007 show that this concept can be used as a pragmatic and very helpful tool for the regulation of genotoxic impurities in new drug substances. It is noted that also the US-FDA’s Center for Drug Evaluation and Research is considering the use of a TTC-based limit for regulation of genotoxic and carcinogenic impurities in drug substances.

4.4.1.2 Staged TTC approach depending on duration of exposure (Müller et al. 2006)

As the TTC value is calculated for a lifetime exposure, higher levels may be also allowed for short-duration treatments. This issue is of relevance for drugs

under development where acceptance criteria need to be adjusted taking into account phase-specific duration of clinical trials as well as the often limited understanding in process chemistry in early phases of development.

Müller et al. (2006) has extended the approach for assessing genotoxic impurities of unknown carcinogenic potential or potency in pharmaceuticals for human use based on the TTC concept as issued by EMA (EMEA 2004, described in section 4.4.1.1) to include the concept of a so-called '***staged***' TTC approach that establishes allowable daily intakes of impurities based upon duration of exposure. The rationale for the suggested approach was that many medicines are given for limited time spans and to limited numbers of patients. Furthermore, exploratory drugs are given in clinical development phases prior to marketing for limited duration under well controlled conditions.

The staged TTC approach presented in Table 8 is based on knowledge about the tumorigenic potency of a wide range of genotoxic carcinogens as well as on the stochastic mode of action (dependency on total cumulative dose as described by Bos et al. 2004). The approach is meant to be used for genotoxic compounds, for which cancer data are limited or not available to determine allowable daily limits for shorter-than-lifetime duration clinical studies.

Table 8. Proposed allowable daily intake (µg/day) for genotoxic impurities of unknown carcinogenic potential during clinical development. A staged TTC approach depending on duration of exposure (allowable daily intakes for shorter durations than 12 months are based on linear extrapolation (Bos et al. 2004) from the TTC value of 0.15 µg/day (Cheeseman et al. 1999, Kroes et al. 2004)). (After Müller et al. 2006).

	Duration of exposure				
	1 month	>1-3 months	>3-6 months	>6-12 months	>12 months
Allowable Daily Intake (µg/day) for different duration of exposure (as normally used in clinical development)	120[a] or 0.5%[c] whichever is lower	40[a] or 0.5%[c] whichever is lower	20[a] or 0.5%[c] whichever is lower	10[a] or 0.5%[c] whichever is lower	1.5[b] [c]

Known carcinogens should have compound-specific risk calculated.
[a] Probability of not exceeding a 10^{-6} risk is 93%.
[b] Probability of not exceeding a 10^{-5} risk is 93%, which considers a 70-year exposure.
[c] Other limits (higher or lower) may be appropriate and the approaches used to identify, qualify, and control ordinary impurities during development should be applied. In particular, approaches that foresee a very low dose of the allowable daily intake ("microdoses") may facilitate higher limits than 0.5%.

As a conservative approach, all exposures >12 months are regarded by the authors as potential lifetime exposures, unless specific arguments are given not to assume this. The delineated allowable daily intake values of between ~1.5 µg/day for ~lifetime intake and ~120 µg/day for ≤ 1 month are considered by the authors as virtually safe doses. The authors also stated that, based on sound scientific reasoning, these virtually safe intake values do not pose an unacceptable risk to either human volunteers or patients at any stage of clinical development and marketing of a pharmaceutical product. They claimed that the intake levels are estimated to give an excess cancer risk of 1 in 100,000 to 1 in a million over a lifetime, and are extremely conservative given the current lifetime cancer risk in the population of over 1 in 4.

The authors have noted that the proposals apply to all clinical routes of administration and to compounds at all stages of clinical development. They also emphasized that it is important to note that certain types of products, such as those for life-threatening indications for which there are no safer

alternatives, allow for special considerations using adaptations of the principles outlined in the paper.
EMA has provided proposals for allowable daily intake for genotoxic impurities during clinical development according to the above-mentioned concept in a Question and Answer (Q&A) document (EMEA 2007, described in section 4.4.1.1). The proposed intakes are 5, 10, 20, and 60 µg/day for duration of exposure of 6-12 months, 3-6 months, 1-3 months, and less than 1 month, respectively. It should be noted that these proposed intakes are half of the values proposed by Müller et al. (2006).
For a single dose an exposure of up to 120 µg is considered acceptable.

According to Humfrey (2007), the EMA guideline based on a TTC derived from animal carcinogenicity data use multiple worst case assumptions to estimate a daily dose of 1.5 µg/day associated with a lifetime cancer risk of 1 in 100,000. Furthermore, based on these assumptions, presentation of the TTC as a single figure infers an unwarranted level of precision and supports the adoption of a more flexible approach by regulatory authorities when evaluating new drug products. Therefore, a range within fivefold of the TTC limit would seem sensible according to the author.
The author also noted that the limit is based on 70 years continuous daily exposure and stated that this is a scenario that is uncommon for most medicines and irrelevant to the preregistration clinical development phase. He expressed the view that the staged TTC approach as outlined in Müller et al. (2006) represents a pragmatic and safety focused approach to the control of genotoxic impurities during the phase of clinical drug development, which has been accepted by some regulatory authorities.

4.4.1.3 An impact analysis of the application of the TTC concept to pharmaceuticals (Delaney 2007)

The recent application of the TTC concept to the regulation of pharmaceuticals in the European Union has recently been analysed (Delaney 2007). The purpose of the paper was to frame the issue of genotoxic impurity limits at a higher level than has been publicly considered by industry and regulatory toxicologists.

According to the author, the reanalysis of the scientific data employed by EMA regulators to rationalise its 1.5 µg/day default genotoxic impurity limit demonstrated 1) that direct translation of conclusions relevant to food consumption are unduly influenced by many classes of potent carcinogens of historic concern which would be impossible to generate unknowingly as pharmaceutical impurities, and 2) that the majority of reactive chemicals that would be useful to synthetic chemists are among the least potent carcinogens in the underpinning supportive analyses.

The author also presented evidence to show that implementation and acceptance of a 1.5 µg/day TTC-based total limit on such impurities can be expected to impede pharmaceutical research and development efficiency while providing an insignificant cancer risk-avoidance benefit to patients who require pharmaceutical treatments.

Finally the author concluded that a significantly higher default limit can readily be defended that would be both in keeping with the TTC concept and the best interest of patients.

The key points established by the author were as follows:

1. In directly applying the TTC concept to pharmaceuticals, the EMA guidance does not take into account the differences in the number of impurities to which an individual can be exposed (a myriad of substances present in food or from food contact materials versus a small number in a pharmaceutical), or the differences in risk equity involved (tradeoff of risk against consequences of remaining untreated to a medical condition, versus the lack of such tradeoffs when being exposed to substances in food).

2. Direct application of the conclusions drawn from Cheeseman et al. (1999, described in section 3.3) and Kroes et al. (2004, described in section 3.4) in the EMA guidance does not take into account the most important contextual difference between food contaminants and pharmaceutical impurities. Reanalysis of the TTC dataset published by Cheeseman et al. (1999) from the perspective of a pharmaceutical process demonstrates that the general perception of carcinogenic potency of DNA-reactive substances is skewed by large numbers of agents that populate several classes representing the highest historical concerns. When discounted for substances purposefully made toxic by a selection process by nature (e.g. aflatoxin B1) or by man (research conducted to identify potent pesticides or antitumor agents), chemicals of concern that a pharmaceutical process chemist might commonly employ as intermediates can be found to array toward the weaker end of the TTC carcinogenic potency spectrum.

3. The extended application of the TTC concept to pharmaceuticals ignores unavoidable risk factors from other sources that put the context of risk avoidance from single sources into proper context. Natural substances are present collectively in foods at substantially higher level than 1.5 µg/day, and those substances generally possess carcinogenic potencies of similar magnitude to substances of current concern used by process chemists to produce active pharmaceutical ingredients (APIs).

4. The existing EMA position ignores the reality that pharmaceutical syntheses frequently require the use of intermediates that are mutagenic in order to be practical.

5. The existence of two disparate regulatory impurity standards, one based on ICH Q3A (ICH 2002) and the other based on the TTC concept, creates a differential cliff of regulatory concern. For a pharmaceutical application to be as effective as intended in creating a “virtual zero risk” standard, the EMA guidance’s TTC-based approach would in effect have to replace ICH Q3A.

Based on these points, the author concluded that expectations to completely avoid mutagenic synthetic intermediates, and to otherwise manage residues below a default limit of 1.5 µg/day are not well supported by deeper analysis of the Cheeseman et al. (1999, described in section 3.3) TTC data, and more recently published scientific knowledge.
As an alterative, a default limit about two orders of magnitude higher can be suggested to meet the “one-in-100,000” risk ceiling deemed acceptable by EMA regulators by using the “hidden buffer” of 10^{-2} to 10^{-3} risk mitigation arising from the conservative assumptions employed in the published derivation of TTC. Such interpretation and use of approximate risk would be scientifically sound and in keeping with the principles of TTC as they were originally developed and published.

To the extent that certain types of impurities require more stringent control (e.g. alkylating agents such as methyl methanesulfonate, or for the rare instance where an impurity aligns closely in structure with a class that's been shown historically to pose high risk), it would seem that an ***in silico*** database primarily based upon carcinogenic potency could be developed and used by industry and regulatory agencies as an alternative to a system that relies solely upon mutagenicity predictions.

4.4.1.4 Multiple genotoxic impurities – cumulative approach (Bercu et al. 2008)

The current available EMA guidance recommends the use of the TTC concept for a single genotoxic impurity where mutagenicity but no carcinogenicity information exists. However, the presence of more than one genotoxic impurity in a new drug substance may occur at trace levels.

Bercu et al. (2008) have, besides repeating the analysis performed by others for a single genotoxic compound, used statistical simulations to assess the impact on cancer risk for a mixture of genotoxic compounds.

Their results for a single genotoxic impurity are consistent with previous analyses (Kroes et al. 2004, Munro et al. 1999). The probability of not exceeding a 10^{-5} excess risk of cancer when exposure is at the TTC value of 1.5 µg/day is 95%, assuming 50% of genotoxic chemicals are also carcinogenic.
Munro et al. (1999, described in section 4.2.1) determined that the probability was 93% for the same dose, excess cancer risk, and assumptions but the database contained "Cohorts of Concern" (COCs), i.e., certain classes of compounds identified as being of higher carcinogenic potency (Kroes et al. 2004, described in section 3.4). Kroes et al. (2004) also observed an increased probability when COCs were removed.
The probability that a genotoxic compound is also a carcinogen (predictive probability) had an impact on excess cancer risk estimation of a single genotoxic impurity. This probability was set at 50% for genotoxic chemicals in risk assessment involving the TTC concept (Müller et al. 2006, described in section 4.4.1.2). As this probability increased, the corresponding cancer risk also increased. For example, for a single impurity, as this probability increased from 10%, 50% to 80%, the cancer risk at the TTC value increased from 1.79×10^{-10}, 3.27×10^{-6}, to 7.71×10^{-6} accordingly.

With the addition of multiple impurities all controlled to the TTC value, an increase in cancer risk was observed.
For multiple genotoxic impurities, the probability that a genotoxic compound is also a carcinogen had a similar effect on the excess cancer risk as it had on a single impurity.
If structurally similar compounds had an assumed strong correlation (±10-fold from the first randomly selected impurity) in cancer potency, the resulting cancer risk was not negatively impacted. For example, if it assumed that 50% of genotoxic chemicals are human carcinogens, then the 90^{th} percentile of the cumulative cancer risk for three impurities at the TTC value would decrease from 1.73×10^{-5} to 1.20×10^{-5}. As the probability that a genotoxic compound is also a carcinogen (predictive probability) has an impact on excess cancer risk estimation of a single genotoxic impurity (increased from 10%, 50% to 80%), the cumulative cancer risk of these impurities at the TTC value increased as well for structurally similar impurities.

In conclusion, with the addition of one to two genotoxic impurities, a slight increase in cancer risk was observed. This increase is, according to the authors, relatively small when considering the conservative assumptions for the TTC concept.
When considering structurally related impurities, the authors advocate that the cumulative cancer risk assessment is more favourable compared to unrelated impurities. This is based on the results of statistical simulation of compounds with related potencies and the expectation that toxicological synergy is not likely at these extremely low doses.
The analysis suggests, according to the authors, that up to three genotoxic impurities, whether structurally related or not, should be acceptable in most cases in pharmaceutical development. Four or more genotoxic impurities is a less likely scenario and should be discussed on a case-by-case basis.

4.4.2 Genotoxic constituents of herbal medicinal products / preparations

The European Medicines Agency (EMA) Committee on Herbal Medicinal Products/preparations (HMPC) has published a guideline on the assessment of genotoxicity of herbal medicinal products/preparations (EMEA 2008), which came into force on 1 December 2008. The Guideline allows using a TTC concept for the risk assessment of herbal preparations containing an identifiable genotoxic compound.

If an established risk assessment method cannot be applied because of the lack of pertinent data, the HMPC suggests using the TTC concept as an option for the assessment of genotoxic constituents in herbal preparations. The HMPC proposes to use the same TTC approach as described in the EMA/CHMP guideline on genotoxic impurities in medicinal products (EMEA 2006, described in section 4.4.1.1), though it is specifically noted by the HMPC that genotoxic constituents in herbal preparations are not considered to be impurities.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR noted that the TTC concept as currently applied is not validated for mixtures and preparations with often variable composition and for which a complete chemical characterisation is often not available. In addition, that plant extracts were not part of the databases used in the derivation of the TTC concept.

5 Discussion on potential applications of the TTC concept

It has been estimated that there are more than five million known man-made chemicals, of which approximately 70,000 are in commercial use (Kroes et al. 2000). There are more than 2,000 high-production-volume chemicals (HPVC, chemicals that are imported/produced in quantities of >1000 tonnes per year), of which a very limited number have been assessed in the EU per year (JRC 2005). In addition, there are 30,000 lower-production-volume chemicals (LPVCs). Furthermore, there are more than 100,000 naturally occurring substances of known structure, and probably many more for which the structure has not yet been elucidated. Little is known about the toxicological properties and human health risks of >95% of these many chemicals and due to the limited resources available for toxicological testing of chemicals it is not realistic to expect that future testing will change this situation significantly. Therefore the TTC concept has been suggested or discussed for application in the risk assessment of chemicals, other than food flavouring agents and food contact materials as well as genotoxic constituents in pharmaceuticals, for which there are no or little toxicological data, but the exposure is expected to be low. This chapter outlines suggested or discussed applications of the TTC concept.

5.1 Industrial chemicals, REACH

The aim of the new European Community Regulation on chemicals and their safe use, the co-called REACH (Registration, Evaluation, and Authorization of CHemicals) is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances.

One of the main reasons for developing and adopting the REACH Regulation 1907/2006/EC (EC 2006b) was that a large number of substances have been manufactured and placed on the market in Europe for many years, sometimes in very high amounts, and yet there is insufficient information on the hazards that they pose to human health and the environment. There is a need to fill these information gaps to ensure that industry is able to assess hazards and risks of the substances, and to identify and implement the risk management measures to protect humans and the environment.

To identify the inherent properties of the substances, information requirements are set out in the testing annexes (Annexes VII-X) that vary according to the tonnage in which the substance is manufactured or imported, and to the needs of the chemical safety assessment. The tonnage ‘trigger’ has been chosen as it gives an indication of the potential for exposure.

Requirements for toxicological information increase when production volume increases and thus, the need to fill the data gaps would result in an increased use of laboratory animals. In order to minimise the number of animal tests, general rules are set out in the REACH Regulation for the use of existing

information in which multiple elements are integrated (including ***in vitro*** tests, read-across, and ***in silico*** approaches i.e., computer models such as (Q)SARs (quantitative structure-activity relationships)), and for waiving of tests (omitting them if they are not required because of their use or it is not technically possible to carry them out). New tests are only required when it is not possible to provide the information in any other permitted way.

The REACH Regulation Annex XI sets out the general rules for adaptation of the standard testing regime (waiving of tests) specified in the information Annexes. Aside from the waiving criteria due to use or technical feasibility, exposure plays a decisive role in the waiving process.
Part 3 of Annex XI deals with substance-tailored exposure-driven testing for sections 8.6 and 8.7 of Annex VIII, Annex IX and Annex X, where, on the basis of the exposure scenario(s) developed in the Chemical Safety Report, testing may be waived. Adequate justification and documentation shall be provided.
In May 2008, ECHA published the technical guidance document on Information Requirements and Chemical Safety Assessment 'REACH TGD' (ECHA 2008a). On the basis of the experience reflected in this guidance document and after consultation of stakeholders, the Commission prepared an amendment of Annex XI stating the criteria for adequate justification for waiving tests, which was adopted on 16 February 2009 (EC 2009).

5.1.1 The TTC concept within REACH

In the guidance document for the implementation of REACH, Chapter R.7C "Endpoint Specific Guidance", Appendix R.7-1 "Threshold of Toxicological Concern (TTC) – a concept in toxicological and environmental risk assessment" (ECHA 2008b), different TTC approaches, their limitations, criteria for use, and finally their potential use under REACH are discussed.

The Nordic Council of Ministers Report 'Application of TTC within REACH' (described in section 5.1.2) as well as the Dutch document produced within RIP 3.3.1 'Information requirements' (described in section 5.1.3) have provided valuable input to the REACH discussion on the potential use of the TTC concept.

Initially in Chapter R.7C it is noted that "***risk assessment for human health effects is based on the threshold of a critical toxicological effect of a chemical, usually derived from animal experiments. Alternatively, a toxicological threshold may also be based on the statistical analysis of the toxicological data of a broad range of structurally-related or even structurally-different chemicals and extrapolation of the no effect doses obtained from the underlying animal experiments for these chemicals to levels considered to be of negligible risk to human health. This latter approach refers to the principle called Threshold of Toxicological Concern (TTC). Regarded in this way the TTC concept could be seen as an extension of such approaches read-across and chemical category.***"

It is also noted that the TTC concept has been developed primarily for use within a risk assessment framework and that the TTC concept is applied for regulatory purposes by the US-FDA and the EU EFSA and UN JMPR[1] in the assessment of food contact articles and flavourings, respectively. These specific TTC approaches underwent a critical review before being accepted on this regulatory platform. Clearly, in the same way, any other TTC

[1] It should be noted that UN JECFA, not UN JMPR, has applied the TTC concept.

approach should be agreed upon by the relevant regulatory body before use, and it should be clearly indicated for which endpoints, routes and population they apply.

5.1.1.1 Limitations

The guidance document points out limitations of the approach with respect to applicability of database, excluded classes of chemicals and extrapolation to other exposure routes than oral exposure:

First of all, they (the TTC values) are derived from data bases covering primarily systemic effects from oral exposure. This is especially important concerning occupational situations where inhalation or dermal exposure is the main route of contact. Only some cover mutagenic, carcinogenic and acute effects, and none (except for the proposed ECETOC approach, described in section 3.6) addresses local effects such as irritation and sensitisation.

As all TTC approaches (except for the proposed ECETOC approach, described in section 3.6) have oral exposure as the principle route, further substantial efforts are needed to explore its potential use for the exposures routes inhalation and skin contact, before any application may become realistic.

Several of the structurally-based approaches to TTC have limitations in applicability domain and cannot accommodate every chemical class. For instance, proteins, heavy metals, polyhalogenated- dibenzodioxins, aflatoxin-like substances, N-nitroso-compounds, alpha-nitro furyl compounds and hydrazins-, triazenes-, azides-, and azoxy-compounds have been excluded by the approach of Kroes et al. (2004, described in section 3.4). Also excluded are highly potent neurotoxicants, organophosphates, genotoxic carcinogens, and proteins.

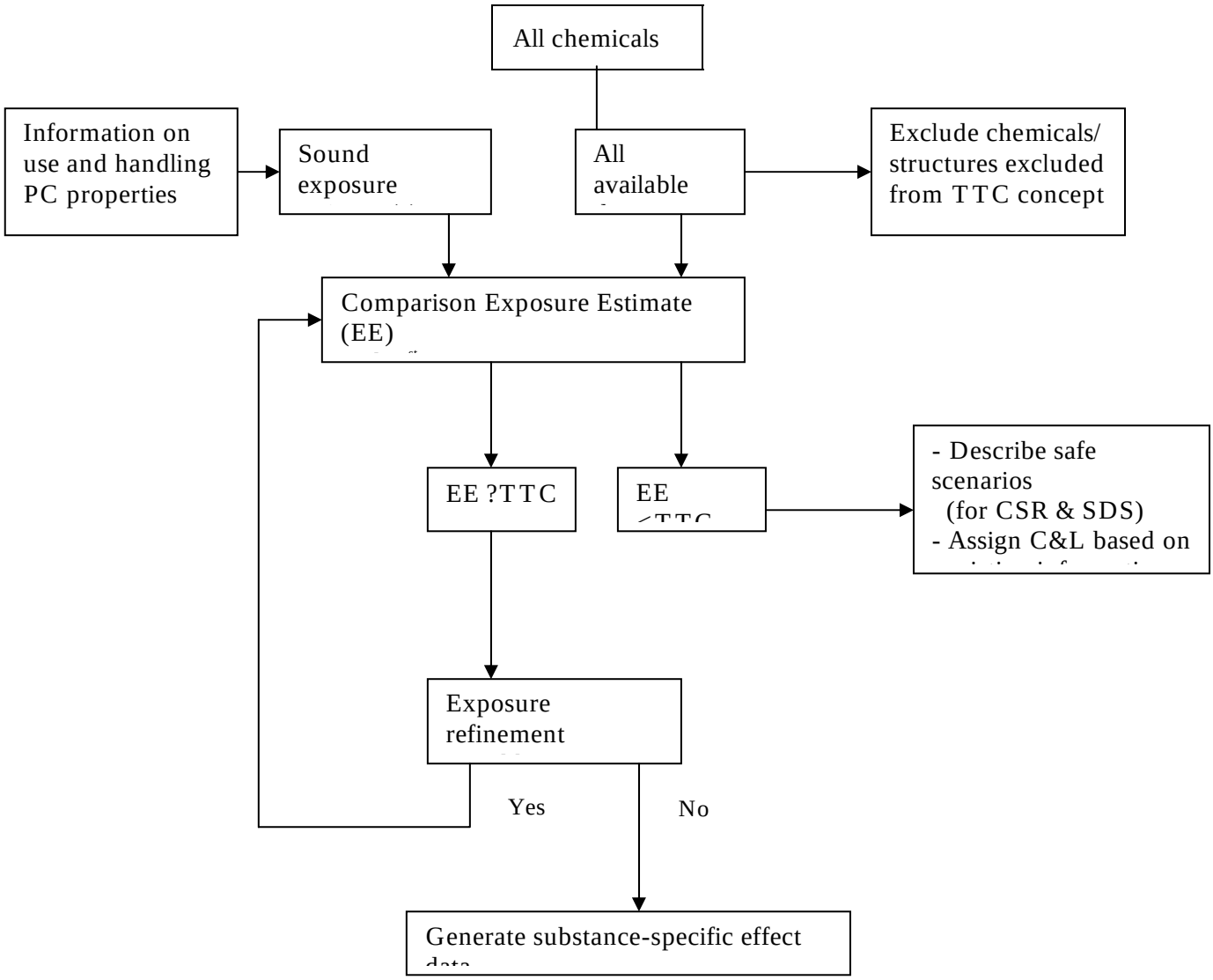
The TTC concept is only applicable in case there is detailed information available on all anticipated uses and use scenarios for which the risk assessment is provided. Based on the experience of the EU Risk Assessment Programme for Existing Substances, robust exposure estimates will require a significant effort, even in cases where the uses were well characterised. In case of a multitude of (dispersive) uses and applications, it may not be feasible to generate overall exposure estimate with detail and precision necessary for use in a risk assessment relying on the thresholds based on the TTC concept. Therefore, a TTC concept will in practice only be applicable in those cases where there are only a few number of exposure scenario's that allow well characterisation.

Finally, the use of the TTC concept does not provide information on classification and labelling of a chemical, or on its potency for a specific effect.

5.1.1.2 Potential uses

Figure 5 illustrates the way a TTC concept can be used. It precedes any chemical-specific testing. One tier is shown, but one could apply additional tiering rounds (as clearly illustrated by the approach presented by Kroes et al. 2004, see Figure 3 in this report) dependent upon the chemical of interest.

Figure 5. Generic TTC scheme/concept under REACH (after ECHA 2008b).



PC properties: Physico-chemical properties
RMM: Risk Management Measures
EE: Exposure Estimate
CSR: Chemical Safety Report
SDS: Safety Data Sheet

Within REACH it is feasible that the TTC concept may be of use for the chemical safety assessment at tonnage levels triggering limited information on repeated dose toxicity and/or reproduction: REACH clearly indicates the need for non-testing methods and provides the opportunity of waiving testing based on exposure considerations. When clearly documented and justified the following options could apply.

Annex VII 'Data requirements for substances at the 1-10 tonnage level':
The testing requirements specified in Annex VII would normally not trigger toxicity testing involving repeated exposures and the information at this tonnage level do provide insufficient information to determine a dose descriptor or any other starting point for the derivation of a Derived No Effect

Level (DNEL) for use in an assessment of the human health risks associated with repeated exposures. Although non-testing or ***in-vitro*** methodologies may give insight in the toxicological properties of a substance, generally such methods are insufficiently specific to provide quantitative information on the potency and/or threshold of an adverse effect. In such a case the threshold derived from the TTC concept might provide a reference value to assess the significance of the human exposure.

Annex VIII-X 'Data requirements for substances at the 10-100, 100-1000 and > 1000 tonnage levels':
At these tonnage levels there may be circumstances triggering an adaptation of the REACH requirements that may lead to waiving of the repeated dose toxicity study and, consequently, the generation of a substance-specific dose descriptor or another starting point for the derivation of a DNEL:
- in Annex VIII, repeated dose toxicity (28-day study) and reproductive/ developmental toxicity (screening study) testing may be waived 'if relevant human exposure can be excluded in accordance with Annex XI section 3'.
- in Annex IX and X testing could be waived in case there is no significant exposure, and there is low toxicity, and no systemic exposure.
In a case-by-case consideration, the appropriate threshold derived from one of the TTC approaches agreed upon by the relevant regulatory body might be considered as a starting point to assess the significance of the human exposure. The level chosen will be critical to ensure a level of sufficient protection.

The guidance document concludes that, independent of the approach used in risk assessment of industrial chemicals, it is important to maintain a sufficient level of protection. In the striving for alternatives to animal testing one suggested approach is the use of generic threshold values. However, application of the TTC concept would imply that limited data may be generated and thus, that the level of protection might be influenced. From information on flavouring substances in the diet the TTC concept seems to be reasonably well based with respect to general toxicity and the particular endpoints examined. However, the possible application of the TTC concept on industrial chemicals needs to be carefully considered. There may be some important differences between industrial chemicals and chemicals used for food contact articles or flavourings, such as differences in use pattern and composition (for a further discussion see NCM 2005, COC 2004).

5.1.2 Nordic Council of Ministers Report: Applicability of the TTC concept within REACH

A report issued by an expert group under the Nordic Council of Ministers (NCM) has evaluated how different TTC-like concepts have been used, and assessed their potential usability in risk assessment of industrial chemicals within REACH (NCM 2005).

The reasons for using the TTC concept within REACH could be to achieve a more effective use of toxicological testing in order to reduce resources and the use of experimental animals. The Nordic expert group considered that if the TTC concept is appropriately derived and used, it might imply a better focus of chemicals at risk. However, the Nordic expert group also stated that independent of the approach used in risk assessment of industrial chemicals it is important to maintain a sufficient level of protection and that application of the TTC concept in REACH would imply that limited data may be generated and thus, that the level of protection might be influenced.

The Nordic expert group noted a number of limitations or drawbacks that should be taken into consideration in deciding whether the TTC concept in general might be applicable for use within REACH:

- In general, the consequences of the assumptions of toxicological, statistical and/or uncertainty factors made in the derivations of the TTC concepts are difficult to overview since there are uncertainties and drawbacks in more or less all of the available TTC approaches.
- A number of studies has been undertaken to investigate whether different endpoints of concern, which might give rise to effects at low doses like immunotoxic, endocrinologic, neurotoxic and developmentally toxic effects could be included in the TTC values. The Nordic expert group considered that not all these endpoints have been adequately covered by the analyses performed today.
- From the investigations performed it has been concluded that all types of substances cannot be included in a concept using TTC; in this respect, the Nordic expert group pointed out that industrial chemicals are diverse and often of complex nature.
- The use pattern of industrial chemicals can often be characterised as wide and dispersive. The Nordic expert group noted that this is different from other groups of chemicals for which the use patterns are considered to be more specific. As an example, the Nordic expert group pointed at food contact materials and flavouring substances.
- Up to now, the TTC concept has only been developed and used for systemic effects following oral exposure (dietary uptake). For industrial chemicals, the predominant exposure is to workers and consumers via inhalation and/or by skin contact. Toxic endpoints of concern for industrial chemicals such as irritation and sensitisation relevant for skin and lung are therefore not covered by the TTC concepts developed up to now.
- Exposure to industrial chemicals includes, in addition to workers and consumers, also man exposed via the environment. TTC values are intended to be used for the general population; however, the Nordic expert group considered that up to now, no considerations to vulnerable subgroups such as children, the elderly and pregnant woman etc. has been made. They also stated that the problem with exposure to the same substance from multiple sources is not solved by the use of the TTC concept.
- The use of the TTC concept is dependent on rather precise quantitative exposure estimates. Experience from the EU Risk Assessment Program for Existing Substances is that it is very difficult to get sufficient information on the different uses and related exposure to make precise exposure estimates. The Nordic expert group considered that for substances where only very limited toxicological data is available, it seems very unlikely that high quality exposure data exist. Furthermore, in relation to industrial chemicals, many different and changing uses of a substance make it very difficult to obtain a robust overall exposure estimate for the substance.
- The Nordic expert group considered that in order to be sure of protective TTC values, the values would be rather small. Using rather crude or conservative exposure estimates (e.g., worst case scenarios and modelling), as is the case for risk characterisation of industrial chemicals, would usually be at a quantitative higher level and thus, this

combination would probably lead to limit the use of the TTC concept to a great extent within REACH.

The Nordic expert group also questioned the TTC concept of generic threshold values based on hazard categories (inclusion in one of three hazard categories based on the hazard classification) proposed by ECETOC (2004) primarily intended to be used in the risk assessment procedure of industrial chemicals within REACH (described in section 3.6 in this report):

- The reasoning for using the classification limit for R48[2] as the numerical starting point for calculating TTC-levels is rather unclear and maybe not especially relevant as a starting point.
- Up to now, there is no experience of hazard categorisation.
- The classification limits are effect values – not 'no-effect' values.
- The use of assessment factors seems rather controversial in this approach.
- There is an obvious risk of misuse if the concept of generic threshold values derived for a specific use (food contact materials and flavourings) is expanded to be used for all kinds of substances, including industrial chemicals, and all possible exposure situations (workers, consumers, and man via the environment). For example, the intended use of GLEV/GEV means use outside the original applicability domain of the concept.

In the EU, testing requirements for chemical substances are proportional to the marketed tonnage levels, described in section 5.1.1 in this report. The Nordic expert group concluded that the TTC concept is not applicable within REACH at marketed tonnage levels below 100 tonnes/year because of the classification requirements and because waiving from testing is possible only at marketed tonnage levels at or above 100 tonnes/year[3].

In the decision whether toxicity studies may be omitted at tonnage levels at or above 100 tonnes/year is appropriate or not, a TTC-value might be used in the comparison with the available exposure information. However, due to limitations and uncertainties in the derivation of TTC-values, as well as the fact that the TTC concept has not yet been evaluated for the diverse group of industrial chemicals and for different routes of exposure other than dietary, the Nordic expert group concluded that it is too premature to use the TTC concept within REACH.

5.1.3 The TTC concept within REACH, Dutch document

In a document produced within RIP 3.3.1-Information requirements (Veenstra and Kroese 2005), the concept of TTC is discussed.

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[2] Generic Lowest Exposure Values (GLEVs) suggested by ECETOC to be used for oral, inhalational and dermal exposure in tiered processes of consumer risk assessment as an estimate of the actual LOAEL for the substance's repeated dose toxicity are based on the classification limit (50 mg/kg bw/day; R48 "Danger of serious damage to health by prolonged exposure"; based on a 90-day study) for repeated dose toxicity according to the Commission Directive 67/548/EC (EEC 1967).

[3] In the form REACH has been adopted in December 2006, exposure based waiving is also possible at the marketed tonnage level of 10-100 tonnes/year with respect to the 28-day repeated dose toxicity test and with respect to the screening test for reproductive/ developmental toxicity, see section 5.1.1 in this report.

It is mentioned that the TTC concept has been incorporated in the risk assessment processes in a number of regulatory schemes as a scientifically sound tool to justify waiving or generation of animal data. It is also stressed that, in contrast to approaches such as read across or chemical categorisation, the use of the TTC concept is not focused or limited to the identification of potential hazards but also provides a quantitative estimate of potency.

It is furthermore noted that the TTC concepts, including the structure-based approaches, are derived from databases covering substances used as direct and indirect food additives, pesticides and industrial chemicals, and cover toxic effects related to systemic exposure to these chemicals. In addition, it is underlined that the TTC concept has not been developed for endpoints associated with direct contact such as irritation or sensitisation.

It is concluded that the TTC concept for systemic toxicity is suitable as a starting point for a tiered testing and risk assessment strategy within REACH. However, it is also underlined that the TTC concepts require a minimum set of information in order to be applied successfully, see below. A draft generic TTC concept under REACH is proposed and it is stressed that the information requirements for the draft generic TTC concept under REACH are consistent with the tiered approach proposed by Kroes et al. (2004, described in section 3.4), i.e. the ILSI concept.

According to the discussion document, the following structural characteristics or properties needs special attention:

- Non-essential, heavy metals and polyhalogenated dibenzo-***p***-dioxins, -dibenzofurans, or -biphenyls and similar substances,
- Genotoxic carcinogens,
- Organophosphates, and
- Proteins.

Taken together, the information necessary for an initial assessment of a substance using the TTC concept is:

- Potential to persist and bioaccumulate,
- Potential for genotoxic carcinogenic action,
- Potential for neurotoxicity and cholinesterase inhibition, and
- Potential for inducing allergies, hypersensitivity, intolerances or local effects.

5.1.4 Applicability of the TTC concept to existing chemicals

A database ‘RepDose’ for repeated dose toxicity data with industrial chemicals has been developed at the Fraunhofer Institute for Toxicology and Experimental Medicine within the framework of the long-range research initiative of the Chemical Industry (CEFIC LRI) (Bitsch et al. 2006).
Data on defined commercial organic chemicals with a limited number of functional groups have been used for constructing the database. Complex and multifunctional chemical structures like pharmaceuticals as well as inorganics, metal compounds and mixtures were excluded. Both oral and inhalation studies with exposure durations from 14 days up to lifetime exposure were entered into the database.
In order to group structurally related compounds, chemicals were characterized by their functional groups. In addition, chemicals were assigned

to categories. Categories include several functional groups and one compound might therefore belong to different categories.
The toxicological effect data include all target organs with all associated effects and corresponding LOELs. Several effects may appear in one target organ at different dose levels and besides the LOELs for the single effects, the overall lowest observed effect level (LOEL) and the overall no observed effect level (NOEL) are included.
In 2006, the database consisted of 364 chemicals investigated in 1018 studies which resulted in 6002 specific effects. The LOELs of all chemicals in the database ranged from 0.006 to 68,907 mg/kg bw per day. Most chemicals have LOELs between 10 and 1000 mg/kg bw per day. (Bitsch et al. 2006).
By November 2009, the database contained over 650 mainly industrial chemicals and also some pesticides tested in repeated-dose toxicity studies with oral and inhalation exposure of rats and mice (Escher et al. 2010).

The applicability of the TTC concept to existing chemicals relevant for REACH has been evaluated by using the RepDose database (Escher et al. 2008a,b, Escher and Mangelsdorf 2009). The RepDose database was compared to the Munro database in order to check whether the threshold values obtained were similar to those suggested by Munro et al. (1996, described in section 3.2). For the determination of TTCs, the lowest LOEL value per chemical in RepDose was compared to those of Munro. As the RepDose database also includes data from inhalation, these data were also included in the analyses.
For the oral route, the number of chemicals in the RepDose and Munro databases was 413 and 463, respectively; for the inhalation route, the number of chemicals in the RepDose database was 213. The number of chemicals in Cramer Classes 1-3 was comparable in the two databases, see Table 9. Most chemicals fell into Cramer Class 3 (60-80%) and Class 1 (17-33%), whereas Cramer Class 2 included only about 3-4% of all chemicals.
For the oral route, the distribution of LOELs was similar in the two databases, although only 95 of the chemicals are identical; however, the RepDose database gave lower values than the Munro database, see Table 9. The distinction of Cramer Class 1 and 3 was quite good in both database indicated by a distance of Cramer Class 1 and 3 of 40 and 55 for the RepDose and Munro databases, respectively. However, in the RepDose database, the LOEL values in Cramer Class 1 were distributed by 5 orders of magnitude (minimum: 0.001 mmol/kg bw/day; maximum: 430 mmol/kg bw/day); 13% of all chemicals in Class 1 had lower values than 0.1 mmol/kg bw/day. The authors expressed the view that a further refinement of Crammer Class 1 or the decision tree seems advisable to achieve a better separation of Class 1 and 3.
By assuming 100% absorption following inhalation lower 5^{th} percentiles were obtained for all data as well as for the individual Cramer Classes, see Table 9. Cramer Class 1 and 3 were less well distinguished for the inhalation route (distance of 3) compared to the oral route.
According to the authors, the analyses confirm that the TTC concept may be applicable to existing chemicals.

Table 9. Analysis of 5th percentiles in the Munro and RepDose databases applicable to threshold derivation (5th percentile values in mmol per kg bw per day). (After Escher et al. 2008b).

	Cramer Class	Ratio Cramer 1 / Cramer 3	N	5th percentiles
RepDose Inhalation	1 2 3	3	71 7 135	0.001 0.003 0.0003
RepDose Oral	1 2 3	40	104 15 294	0.04 0.007 0.001
Munro	1 2 3	55	78 14 371	0.11 0.03 0.002

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR noted that for the oral route the RepDose database gives lower values than the Munro database for all 3 Cramer classes and that the number of Cramer Class II chemicals is very small in both databases. The DG SANCO Expert Group also noted that a major overlap of NOELs and LOELs between Cramer Class I, II and III was demonstrated and that a better separation of the Cramer classes is needed.

5.1.5 Exposure-triggered toxicity testing under REACH: Use of the TTC concept to define significant/relevant exposure (Bernauer et al. 2008)

As mentioned in the introduction to section 5.1, exemption from conducting individual toxicity tests (waiving) is possible in cases where exposure is to be neglected (Annexes VIII-XI). However, it is difficult to define what constitutes “no exposure” and the REACH Annexes VIII-XI use different terms to describe the conditions that allow waiving based on exposure considerations (“no relevant exposure”, “limited exposure”, “no exposure”, “no significant exposure” and “unlikely exposure”), and criteria are lacking which precisely define these terms. Attempts have been made to establish cut-off criteria between “non-relevant” and “relevant” (detrimental) exposure based on external exposure concentrations and the TTC concept.

The recent paper by Bernauer et al. (2008) make a proposal and describe a strategy how to define the currently insufficiently described terms “relevant/significant” exposure.

The authors proposed to define relevant/significant exposure based on an endpoint-specific TTC approach, starting from a comparison of the tentative external exposure to the specific TTC value. This can be followed by a refinement of exposure estimates and may culminate in the experimental determination of internal and target tissue exposure. This strategy enables a well-founded assessment of what “no relevant exposure” is and safeguards an appropriate level of protection of the general population.

For the establishment of endpoint-specific TTC values, existing data on NOAEL or NOAEC values for specific endpoints can be used to determine their empirical distribution and to define a cut-off value. This has been demonstrated in the paper for fertility and developmental toxicity by Bernauer et al. (2008), described in section 5.10.2). However, Bernauer et al. (2008) have noted, with a reference to Barlow (2005, described in section 3.4) that there are limitations in databases for certain endpoints such as allergenicity or endocrine disruption.

The principle of the approach to separate relevant from non-relevant exposures consists of several steps. In the first step, external exposure is estimated and its extent is compared to an endpoint-specific TTC value. Thus, this procedure relies on external exposure concentrations. However, internal exposure represents a more important metric than external exposure, in particular for systemically induced effects. Therefore, where necessary, it might be appropriate to refine exposure assessment by determining internal exposure.

The authors stated that their proposed strategy holds the opportunity for the protection of the population from the adverse effects of chemicals and at the same time for avoiding unnecessary testing and thus respecting animal protection.

5.2 Chemicals in food

The mandate for an EFSA SC Working Group on Threshold of Toxicological Concern (TTC) was adopted at the 31st plenary meeting of the EFSA Scientific Committee (EFSA 2008). The mandate refers to the TTC concept as the establishment of a generic human exposure threshold value below which there would be no appreciable risk to human health. This concept has been used by the EFSA's former AFC Panel for the safety assessment of food flavourings. The purpose of the new mandate is to look at a possible broader applicability of the TTC concept in other areas of risk assessment performed by EFSA Scientific Panels. (DG SANCO 2008).

5.2.1 Food additives

Food additives are substances that are added intentionally to foods to perform certain technological functions. In the EU, food additives are authorised by Regulation (EC) No 1331/2008 (EC 2008c) on a common authorisation procedure for food additives, food enzymes and food flavourings and Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives (EC 2008d). The Regulations entered into force on 20 January 2009. These regulations replace a framework directive (EEC 1988a) and three specific directives on colours (EC 1994a), sweeteners (EC 1994b) and the remaining food additives (EC1995).

Prior to their authorization, food additives are evaluated for their safety. According to the regulation only additives that have undergone a full toxicological evaluation are authorized. Therefore, the TTC concept is not considered relevant so far in the risk assessment of the food additives (DG SANCO 2008).

The ILSI Expert Group which evaluated the TTC concept for chemical substances present in the diet recommended that the TTC concept can be used for substances that are present in food in low concentrations, which lack toxicity data, but for which an exposure assessment can provide reliable exposure estimates (Kroes et al. 2004, described in section 3.4).

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have concluded that the TTC concept may possibly be used if an unsuspected chemical or impurity be detected in a food additive. In future, other areas of risk assessment may be included.

5.2.2 Food contaminants

The ILSI Expert Group which developed and evaluated the TTC concept for chemical substances present in the diet recommended that the TTC concept can be used for substances that are present in food in low concentrations, which lack toxicity data, but for which exposure assessment can provide reliable exposure estimates (Kroes et al. 2004, described in section 3.4).

5.2.3 The TTC concept as a screening and prioritising tool for chemicals in food: The Institute of Food Technologists view (IFT 2009)

A group of experts, convened by the Institute of Food Technologists (IFT) has examined the complexities that challenge timely decision-making about the presence of undesirable chemical contaminant or unanticipated chemical substance in a food commodity, ingredient, or finished product when available scientific information is limited and defined and developed a workable tool to guide food safety managers in effectively and knowledgeably evaluating available scientific evidence pertinent to assessing the risk from exposure to a chemical substance to make timely decisions.

The group considered a TTC concept based evaluation to be an efficient screening and prioritising tool which might lead to a decision that, for some chemicals, further research and risk mitigation steps are necessary while for others, further research is not necessary and a decision about risk is possible.

A 'toxicological priority grid' was developed based on three categories of priority ranking: Low, medium or high toxicological priority.

The grid incorporates toxicological potency ranked into low, medium or high (based on experimental data if available or on structure activity relationships (SAR)) and potential consumer exposure ranked into low exposure (less than the TTC value of 1.5 µg/day), medium exposure (1.5-4.5 µg/day) and high exposure (above 4.5 µg/day). The approach also incorporates two sets of food consumption data that will allow rapid determination of the range of exposure: (1) a set for substances of concern that are believed to be present in only one or a small number of foods, and (2) another set for substances believed to be present in commodity ingredients that are components of a large number of different foods. On average, adult humans are assumed to consume 3 kg of foods and beverages (including water) per day.

In order to interpret the evaluation the group provides the following explanations:

The low priorities (little or no safety issue) correspond to either a low or medium exposure to a substance with a low to medium order of structural activity and acute toxicity. Typically, one would defer to the toxicity profile when available and to the SARs in situations for which only minimal toxicological data are available. Situations occurring within the green squares allow a recommendation of a low priority concern, and little follow-up work is indicated.

The medium priorities correspond to high/low, medium/medium, or low/high exposure/toxicity combinations. Typically, one would defer to the toxicity profile when available and to the SARs in instances of only minimal toxicological data. Because the exposure level and/or toxicity profile is higher

in this situation, it is critical that the decision maker have confidence in making sound recommendations to those involved in risk management. Situations involving the yellow squares will often constitute the most difficult decisions that a food safety professional will have to make, based on limited analytical and toxicological data.
Medium priority concerns indicate the need for additional information to allow a risk assessment to occur. Information about potential exposure and toxicological effects is necessary to determine the scope of the issue and source. Toxicological analysis would assure a full and complete understanding of the existing data set that is available. Consideration of the source of the exposure as per ingredients, commodities, and food products is essential.

The high priorities correspond to combinations of high or medium exposures with high or medium toxicity. Typically, for high-priority issues, only interim risk management decisions can be made until traditional, full-scale information is available on toxicity and exposure. Because the exposure level and/or toxicity profile is higher in this situation, the decision maker must have complete confidence in making recommendations to senior officials. Red-square situations often constitute the most clear-cut decisions that a food safety professional has to make, based on limited analytical and toxicological data. High priority concerns receive the highest level of immediate attention and risk management response. As with green and yellow situations, one should consider the source of the exposure as per ingredient, commodities, and whole foods.

5.2.4 Refining the TTC concept for risk prioritisation of trace chemicals in food (Felter et al. 2009)

A recently published paper (Felter et al. 2009) emphasized the need for putting low-level detections of unintended chemicals in food in the context of safety assurance, using the TTC concept as a tool to facilitate prioritisation of responses and resource allocation. The authors have suggested that the TTC approach would be employed as a 'screening' assessment to allow risk managers to make rapid, scientifically defensible, consistent, and transparent decisions as to the urgency of responses needed to address the discovery of unexpected chemicals in food. They also emphasized that the TTC limit is a conservative screening tool and that exposures exceeding this level are not necessarily associated with any health concerns but rather are flagged as warranting further evaluation.

The paper describes two areas in which the established TTC concept can be modified to increase its utility for addressing issues often associated with unintended chemicals in food.
First is the refinement of the TTC-based decision tree published by Kroes et al. (2004, described in section 3.4) to allow for the inclusion of Ames data on chemicals with structural alerts for genotoxicity. This refinement is justified by the authors as Ames data are often the only data available in the publicly available literature or easily generated for newly identified chemical contaminants, and therefore, it is important that the tiered approach offers a way to integrate these data into the appropriate TTC-based exposure tier.
Second is the establishment of an approach that recognizes that higher limits can be established for short-term exposures. The authors have stated that the TTC concept was originally developed as a tool to evaluate potentially chronic exposures arising from food packaging materials, and as such was based on lifetime toxicity studies in rodents. But as human exposure to unintended chemicals in food is often only for a limited time and accordingly,

the authors found that the exposure limits can be modified to address short-term or intermittent exposures.
The TTC tiers resulting from the incorporation of Ames data and/or shorter duration exposures are summarized in Table 10. The authors have noted that there may be situations where a specific chemical has structural alerts and negative Ames data, and exposure is expected to be less than 1 year. Specific guidance is not offered for this scenario, but the authors have recommended that this be handled on a case-by-case basis, dependent on the amount and quality of data available.

Table 10. Proposed short-term exposure thresholds for potentially genotoxic contaminants in food (After Felter et al. 2009).

	Lifetime daily exposure	Exposure expected not to exceed 1 year
Chemicals with structural alerts for genotoxicity	0.15 µg/day	1.5 µg/day
Chemicals with structural alerts, but negative Ames data [a)]	1.5 µg/day	Case-by-case

a) Or other data sufficient to conclude a lack of DNA reactivity

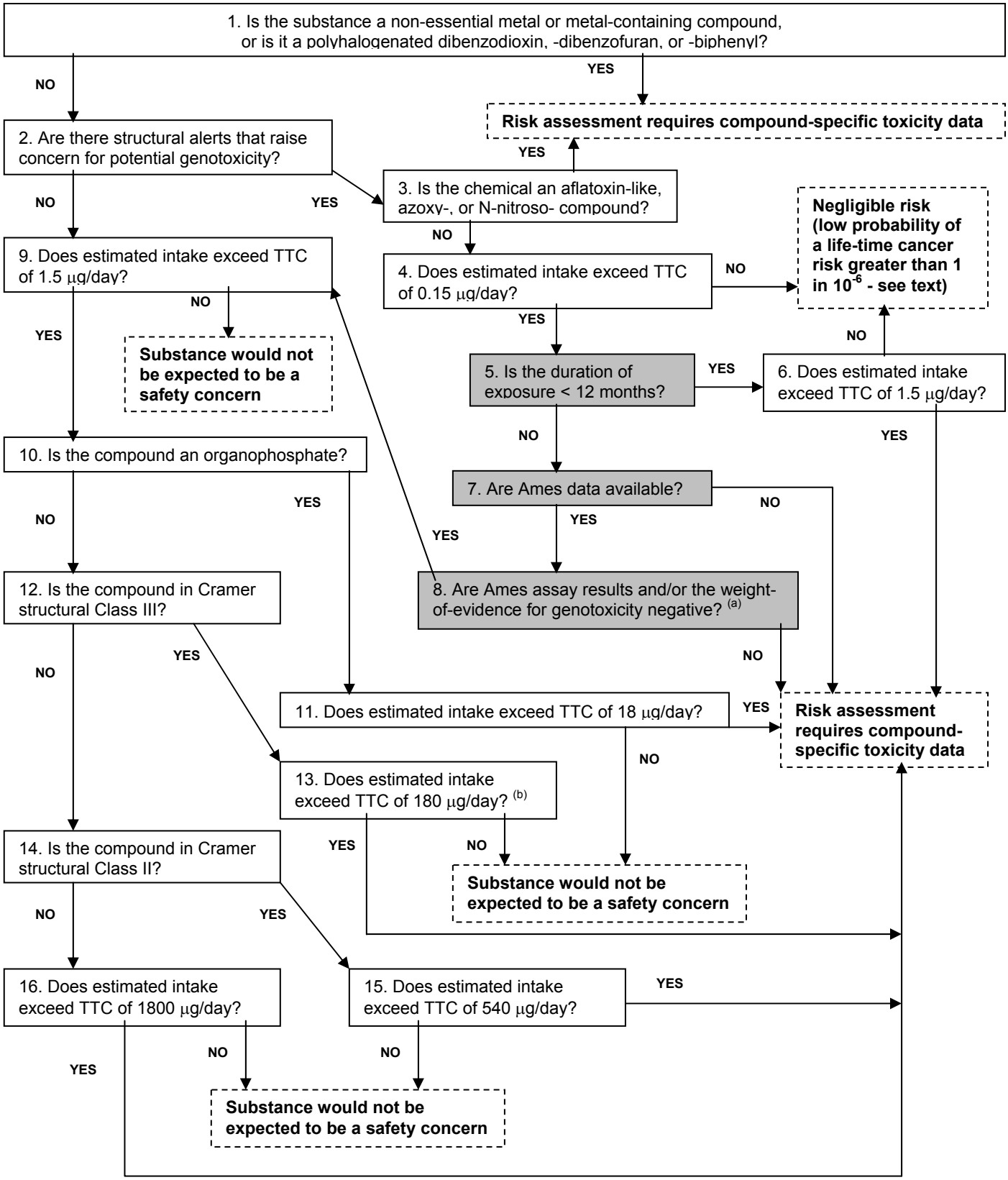
The paper proposes a decision tree for risk prioritization of chemicals in food, see
Figure 6. The tiered TTC flow diagram published by Kroes et al. (2004), and recently refined by Munro et al. (2008), serves as the initial basis for evaluating low-level contaminants in food. Munro et al. (2008) reanalyzed the data for Cramer Class III without organophosphates (since they are treated separately and assigned a TTC value of 18 µg/day in the decision tree) and concluded that the Class III TTC tier should be increased from 90 to 180 µg/day.

5.2.4.1 Refinement of the TTC concept to allow for the inclusion of Ames data on chemicals with structural alerts for genotoxicity

Consistent with Kroes et al. (2004, described in section 3.4), the first step is to exclude certain chemical groups such as proteins, heavy metals, high-potency carcinogens, and polyhalogenated dibenzo-p-dioxins, -dibenzofurans and -biphenyls from using the TTC concept to support an acceptable exposure limit.
For the high-potency carcinogens such as aflatoxin-like, azoxy-, and N-nitroso compounds the authors have emphasized that these chemicals are excluded because there has not been a sufficient analysis of the potency of these chemicals to support an appropriate TTC-based exposure tier, but that it is likely that the principle of TTC can still be applied once a sufficiently robust evaluation has been done. They also find that the exposure limit for these high-potency carcinogens would likely be significantly lower than the current limit of 0.15 µg/day for genotoxic chemicals in general.

The second step is to determine whether the chemical contains structural alerts for genotoxicity. For these chemicals the current limit is 0.15 µg/day. According to the authors, the Kroes et al. (2004, described in section 3.4) decision tree does not explicitly address how to handle chemicals with structural alerts (raising a concern for possible genotoxicity) but also with negative genotoxicity data.
Figure 6. Proposed decision tree for risk prioritisation of chemicals in food. Shaded boxes indicate additions made to the Kroes et al. (2004) decision tree. (a) This refers to the results of an Ames Bacterial Mutagenicity Assay conducted according to internationally accepted protocols (e.g., OECD). In addition to Ames, all available

genotoxicity data should be considered in this determination. (b) This threshold has been increased to 180 μg/day from the 90 μg/day specified in the original Kroes et al.(2004) decision tree, based on the new analysis of Cramer Class III by Munro et al. (2008). (After Felter et al. 2009).



The range of carcinogenic potencies (e.g., TD50s in the CPDB, cancer slope factors in US-EPA's IRIS database) spans at least six orders of magnitude. The authors have stated that this emphasizes the conservative nature of using a single TTC-based exposure limit that has been established to be protective for the vast majority of these carcinogens (i.e., 0.15 µg/day) for any compound with structural alerts that suggests the potential for it to be a carcinogen. And they find that this represents an opportunity to further refine the TTC approach if there are additional data that can be considered.

For chemicals with structural alerts for genotoxicity, but for which there are negative Ames data the authors have proposed that a separate tier of 1.5 µg/day be established. The authors found that the most relevant work was that of Cheeseman et al. (1999, described in section 3.3) who analysed 709 carcinogens in the CPDB to determine whether specific criteria could reliably predict whether an untested substance is likely to be a potent carcinogen, if it is later found to be a carcinogen at all. Cheeseman et al. (1999) examined the use of short-term toxicity data (i.e., LD-50 data), the results of genotoxicity testing and structural alerts to identify potent and non-potent subsets of the 709 carcinogens. The potent subsets could then be used to provide the basis for excluding substances from the Threshold of Regulation (TR) process, or to restrict their use to dietary concentrations at or below 0.5 ppb. Similarly, the non-potent subset could be used to establish higher thresholds for untested substances that are likely to be less potent carcinogens.
As part of this evaluation, Cheeseman et al. (1999) determined that substances testing negative in the Ames test have cancer potency about an order of magnitude lower than Ames positive carcinogens. They concluded that a dietary level of 4-5 ppb (equating to an intake of 12-14 µg/day) would be protective for chemicals with structural alerts that have tested negative in the Ames assay.
Based on the analyses by Cheeseman et al. (1999), the authors concluded that the assignment of a TTC-based exposure limit of 1.5 µg/day for chemicals with structural alerts, but negative Ames data is a conservative first step in the inclusion of genotoxicity data in the tiered TTC approach. Additional refinements may be achieved pending further analyses of additional genotoxicity/mutagenicity assays (and combinations thereof) as they relate to carcinogenic potency.

On the other hand, the decision tree published by Kroes et al. (2004) and modified by Munro et al. (2008) should be used for chemicals without structural alerts. These include tiers for organophosphates and chemicals classified into each of the three Cramer Classes based on their chemical structure. The basis for and application of these non-cancer TTC tiers in risk assessment has been described extensively in section 3.4 and presented in Figure 3.

5.2.4.2 Expanding the TTC concept for short-term exposures

Felter et al. have noted that the TTC exposure limits that have been described for cancer and non-cancer endpoints were set at a level that assumed a potential for lifetime human exposure. But as many chemicals that are found as unintended contaminants in food are likely to be present for only a short period of time, the authors found that the application of TTC-based limits that are considered to be protective for a lifetime of exposure would be conservative in these cases and therefore, presenting an opportunity for refinement of the assessment.

According to the authors, 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, described in section 5.5.1) for evaluating exposures associated with cosmetics that are not used on a daily basis. The authors have noted that while this is a valid approach, it also requires that fairly robust data on the nature of the exposure and its duration are available.
A second approach would be to establish TTC-based limits for short-term exposure durations that are less well-defined as suggested by Müller et al. (2006, described in section 4.4.1.2) to establish different TTC tiers for contaminants in pharmaceuticals corresponding to different exposure durations.

According to the authors, an approach similar to that suggested by Müller et al. (2006, described in section 4.4.1.2) could be used for contaminants in foods.
The authors have proposed that for chemicals that would be assigned a TTC limit of 0.15 µg/day based on the presence of structural alerts for genotoxicity, a corresponding limit for short-term (e.g., up to 12 months) exposures could be established at 1.5 µg/day. The basis for this recommendation came from the use of lifetime cumulative dose (LCD) as the appropriate dose metric for carcinogens that have been assessed using linear extrapolation methods. The concept of cumulative dose as a dose metric in toxicology states that an effect is the result of total dose over a period of time, such that a higher daily exposure for a shorter time period is toxicologically equivalent to a lower daily exposure over a longer period of time (Haber's law). The authors recognized that this is a simplification of a much more complex relationship between dose and time, and expected that dose-dependent transitions in mechanism may be such that risk could be over- or under-estimated using this approach. They also recognized that, from a cancer theory perspective, it can be anticipated that by using the cumulative lifetime average dose as the dose metric for shorter duration, higher dose rate exposures will either under-predict or over-predict risk depending on which stages (if any) are dose-rate dependant and at what time the exposure occurs.
The authors have noted that both theoretical arguments and empirical observations suggest that the degree to which lifetime average dose may underestimate risk is relatively modest. It has been proposed that using lifetime average dose could theoretically under-predict risk by about an order of magnitude, which is consistent with the results of empirical studies. It can be anticipated that the more the dose is compressed (the shorter the time period within which the cumulative lifetime dose is administered and therefore the higher the dose rate), the greater the possibility that risk will be underestimated.

The authors have mentioned that the underlying assumption of LCD as the most appropriate dose metric to assess cancer risk is described in Section 3.4 of the US-EPA 2005 cancer risk assessment guidelines (US-EPA 2005). They have also noted that in the ECHA (2008a) guidance for the implementation of REACH (REACH TGD), there is acknowledgement that higher exposures to carcinogens for less-than-lifetime exposures can be tolerated. Specifically for the risk assessment of genotoxic carcinogens presumed to have no threshold, it is recommended that a factor of 2.8 be applied to risk assessments for workers who are exposed for a fraction of

lifetime (2.8 is based on an assumption of occupational exposure of 5 days/week, 48 weeks/year and 40/75 years) (ECHA 2008a). According to the authors, this means that a daily lifetime exposure limit for the general population is considered to be toxicologically equivalent to a daily workplace exposure that is 2.8-fold higher on a per day basis (but equivalent on the basis of total exposure over a lifetime).
The authors have noted that the recommendation presented in the paper (an additional TTC tier of 1.5 µg/day be established for short-term exposures, i.e., less than 1 year) to chemicals with structural alerts represents a first screen and thus is intended to be conservative. In this respect, they have noted that the use of the LCD would suggest that the daily exposure for a 1-year duration could be 70-times higher than the lifetime average daily dose (LADD). And even if a 2- to 5-fold factor (as described by in US-EPA 2005 cancer risk assessment guidelines) should be incorporated for uncertainty associated with this averaging approach, the daily exposure for a 1-year duration could still be 14- to 35-times higher than the LADD.
Therefore, the authors concluded that the increase of one order of magnitude (from
0.15 µg/day to 1.5 µg/day) for exposures not exceeding 1 year is clearly a conservative approach.

The authors also have noted that where more robust exposure data are available, it might also be appropriate to adjust the exposure data to determine an average daily dose, which could then be compared with the original TTC values. They also expected that additional research into this area may result in guidance that can be further expanded into various TTC tier thresholds based on dose duration as Müller et al. (2006, described in section 4.4.1.2) suggested for pharmaceutical impurities. Similarly, that further work in this area may also lead to expanded guidance for short-term exposures to non-genotoxic chemicals, i.e., those for which TTC-based exposure limits are based on the Cramer classifications.

5.3 Veterinary drug residues in food of animal origin

Residues of veterinary medicinal product in food commodities are assessed in accordance with Commission Regulation (EEC) 2377/90 (EEC 1990).
In order to use a veterinary drug in a food animal in any country within EU the pharmacologically active substances included in the medicinal product have to be listed in either annex I, II or III of the Regulation. Annex I contains pharmacologically active substances for which an MRL has been approved, and Annex III contains pharmacologically active substances for which a provisional MRL has been set. Substances, for which it appears that it is not necessary to establish a maximum residue limit for the protection of public health, are included in Annex II. Annex IV contains prohibited substances. (VKM 2006).

Within EMA, it is the Committee for Medicinal Products for Veterinary Use (CVMP) that performs risk assessment of veterinary drug residues in foods following the use of veterinary drugs in food animals. Currently, the CVMP does only use ADI as the reference limit in the risk assessment of residues and for the derivation of maximum residue levels (MRL). However, according to the CVMP Safety Working Party (SWP) work programme for 2006, the SWP is going to consider alternative reference limits. (VKM 2006).

One area of application of the TTC concept could be for veterinary drug preparations with limited sales potential, often referred to as “orphan drugs”. Such drugs used for the treatment of rare diseases and against intoxications are usually prepared ***extemporaneously*** (mostly by a pharmacy) from a veterinary recipe and are not evaluated by EMA and thus, not included in any of the annexes of the Regulation 2377/90. (VKM 2006).

Another area of potential use is the assessment of low level dietary exposure scenarios, in particular those resulting from residues in food producing animals from use of substances of botanical and homeopathic origin. In the past, the CVMP has applied an “exposure-driven” hazard characterization; a pragmatic TTC-like approach based on the assumption that exposure to residues of individual constituents would be too low to present a significant risk to consumers. Based on this, homeopathic preparations of D4 (dilution 1:10000) and higher got an entry in Annex II (“List of substances not subject to maximum residue levels (MRL)”) of the Regulation 2377/90 without further in-depth toxicological evaluation of residues. (DG SANCO 2008).

The TTC concept may also be of benefit in the evaluation of potential health risks from certain impurities in pharmaceutical formulations. For human medicinal products a TTC concept has been adopted for genotoxic impurities (described in section 4.4.1.1). There is ongoing discussion to introduce such a limit for veterinary medicinal products (DG SANCO 2008).

The CVMP has noted in its discussions, that TTC values do not exist for all relevant toxicological endpoints and that several TTC values are still at an exploratory stage requiring further in-depth examination. In addition, it was emphasized that in the further development of the TTC concept adequate attention needs to be given to those aspects that are specific to active compounds as those used in veterinary medicines. In this area endpoints for pharmacological and microbiological effects play a quantitative important role in the evaluation of exposure scenarios. It appears that none of these endpoints currently fit into the effect categories for which TTC values have already been elaborated. (DG SANCO 2008).

On the international level the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) adopts international MRL values, so-called Codex MRLs, based on scientific expert advice provided by the JECFA. So far, Codex has adopted MRLs for residues of approximately 50 veterinary drugs. However, it is estimated that JECFA has evaluated less than one third of the pharmacological substances used in food animals worldwide. Many medical substances with the potential to leave residues in foods have no ADI and no international MRL. A working group under CCRVDF has recommended that for those substances for which it is not possible or practical to establish an ADI or a MRL, Codex should work in conjunction with JECFA to consider alternative risk assessment tools such as margin of exposure, threshold of toxicological concern, and statistical approaches. (VKM 2006).

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have noted that the TTC concept is currently not used in the assessment of consumer safety of residues of veterinary medicinal products in food, but that the TTC concept might offer an appropriate option in the assessment of substances that have no ADI/MRL and certain impurities/trace level residue concentrations. It is also to be noted that

endpoints like pharmacological or microbiological effects are not addressed in the currently available databases.

5.4 Drinking water contaminants and materials intended for contact with drinking water

According to the EU Drinking Water directive 98/83/EC (EC 1998a), Member States shall lay down the parametric values corresponding at least to the values set out in the Directive. Where parameters are not set out in the Directive limit values must be laid down by the Member States if necessary to protect health. (DG SANCO 2008).

At present, very different national systems exist in Europe for risk assessment and approval of products and/or materials intended for use in contact with drinking water (DG SANCO 2008, VKM 2006).
It is the manufacturers and/or the importers of such products who are responsible for producing the products according to good manufacturing practice and for the quality of the products. The content of impurities and the migration of components directly or indirectly from such products to drinking water should be as low as technically possible. Furthermore, the migrating substances should not pose any risk to human health. (VKM 2006).
Development of a common system for approval of materials intended for use in contact with drinking water within EU, called the European Acceptance Scheme (EAS), has been going on since 1999. Because these materials are defined as construction products, this work is based upon both the Construction Products Directive (89/106/EEC) (EEC 1988b) and the Drinking Water Directive (98/83/EC) (EC 1998a). At the moment, it is not clear when EAS will be adopted and most likely replace the various existing national systems of approval in Europe. (VKM 2006, DG SANCO 2008).

Within EAS, the list for plastic food contact materials developed by SCF/EFSA, has been used as a starting point for making a positive list of plastic materials that can be used in contact with drinking water (VKM 2006, DG SANCO 2008).
In the evaluation of food contact materials migration thresholds are used to decide the amount of toxicity data needed to be supplied by the petitioner to the AFC Panel in EFSA (described in section 4.1.2). It is quite possible that this approach will be employed to be able to assess and approve all the numerous products that are used in contact with drinking water, when the EAS process is more mature. In addition to the use of reduced packages of toxicity data according to preset migration limits, a TTC limit below which no toxicity data is required has also been discussed within EAS. A threshold of 0.1 µg/l, based on limits for the genotoxic substances acrylamide and epichlorohydrin in the Drinking Water Directive (98/83/EC) (EC 1998a), has been considered. (VKM 2006).

An ILSI Europe workshop held in 1998 suggested that the TTC concept should be developed to facilitate progress in risk assessment for drinking water contaminants (ILSI 2002). A scientific judgement should be made as to what level of contamination in drinking water represents a threshold of toxicological concern, and any contaminants found below such a threshold level would be considered to have low priority for risk assessment or monitoring. Such an approach would leave a manageable number of chemicals for further consideration. As an example, they stated that the large number of pesticides found in raw waters would be unlikely to remain as

priority chemicals under such a scheme since they are generally present at such low levels. (VKM 2006).

In the USA, the Safe Drinking Water Act (SDWA) governs the quality of drinking water. Under SDWA, the United States Environmental Protection Agency (US-EPA) sets national health-based standards for drinking water to protect against both naturally occurring and man-made contaminants that may be found in drinking water. It is the NSF International (previously called The National Sanitation Foundation), an independent, private, non-profit, third party organization, which do certification, testing and write standards for products, materials, and systems in connection with drinking water in USA. It thus certifies direct water additives (drinking water treatment chemicals) and indirect additives (drinking water system components). The method of risk assessment used by NSF International is determined by the quality and quantity of toxicity data available for the product component under evaluation. When the available toxicological data is insufficient to perform either a qualitative or a quantitative risk assessment, or when toxicological data is available, but the normalized contaminant concentration does not exceed the applicable "threshold of evaluation" value, a qualitative review of the available data shall be performed to determine whether adverse health effects can result at the threshold of evaluation concentration. This threshold of evaluation concept is based on the TTC/Threshold of Regulation concept. At present, two levels are used for chronic exposure. Toxicity testing is not required for a substance having a normalized concentration of 3 µg/l during static normalization conditions, or 0.3 µg/l during flowing normalization conditions. For short-term exposure, if a short-term toxic effect is not identified by the available data, the initial (day 1) laboratory concentration shall not exceed 10 µg/l. These thresholds of evaluation values should not be applied to substances for which available toxicity information indicate that adverse human health effect may result from the use. If the normalized contaminant concentrations exceed the threshold of evaluation concentrations, it is possible to perform a chemical class-based evaluation of the substances on the basis of the known toxicities of other chemicals of similar structure and functionality. The current use of the threshold of evaluation concept is intended to be expanded to include multiple levels, and at present six levels are proposed. (VKM 2006).

5.5 Cosmetic products

The safety in use of cosmetic products has been established in Europe by controlling the ingredients, their chemical structures, toxicity profiles, and exposure patterns as set out in Council Directive 76/768/EEC (EEC 1976) and its amendments.

The safety of a cosmetic product in the EU is the full responsibility of the manufacturer, the first importer into the EU market, or the person placing the product on the market. The safety of a cosmetic product is based on the safety of its ingredients, the latter being evaluated by toxicological testing.

Two channels function with respect to the safety evaluation of cosmetic ingredients in the EU. The Scientific Committee on Consumer Safety (SCCS) is responsible for the safety evaluation of the cosmetic ingredients listed in the Annexes (II, III, IV, VI and VII) to Directive 76/768/EEC (EEC 1976) whereas the safety evaluation of all ingredients of cosmetic products

other than those of the Annexes, is the responsibility of the manufacturer through the safety assessor (SCCP 2006).
The Scientific Committee on Consumer Products (SCCP, now the SCCS) has adopted 'Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation' (SCCP 2006). The Notes of Guidance was designed to provide guidance to public authorities and cosmetic industry, in order to improve harmonized compliance with Directive 76/768/EEC (EEC 1976) and in particular by the 6th (Directive 93/35/EEC) (EEC 1993) and 7th (Directive 2003/15/EC) (EC 2003) Amendments to this Directive (SCCP 2006).

The Notes of Guidance include a full evaluation of the effects of a chemical on all toxicological endpoints.
The calculation of exposure to a cosmetic ingredient is based on the specific use of the product(s) containing the ingredient, taking into account the application method, concentrations in the products, amount used per application, frequency of application, body area of application, contact time etc. The specific exposure scenario is translated into a systemic exposure dose (SED, the amount expected to enter the blood stream, expressed in mg/kg bw per day). All relevant ways of exposure (dermal, oral and/or inhalation) are taken into account depending on the particular product/ingredient.
The last step in the risk characterization is to divide the lowest NO(A)EL value obtained in an animal experiment with SED to obtain the margin of safety (MOS). MOS should be at least 100 to declare a substance safe for use.

A TTC approach is currently not applied in the safety evaluations of cosmetic ingredients. However, the TTC concept is mentioned in the Notes of Guidance and it is noted that the SCCP is closely following up discussions on the concept of the TTC (SCCP 2006). The possible use of the TTC concept for the safety evaluation of cosmetic ingredients has been discussed in a COLIPA Expert Group and reported by Kroes et al. (2007, described in section 5.5.1).

According to Bridges (2003) there are about 8000 cosmetic ingredients listed in the Blue List (2001) and even more in the International Nomenclature of Cosmetic Ingredients (INCI) list. Only about 5% of these have been evaluated for their effects on human health.
As it can be foreseen that the regulatory requirements for cosmetics and personal care products will be tightened in the future more rapid and efficient methods for risk assessment, such as the TTC concept, will be needed. However, the databases used to develop the TTC concept comprise experiments using oral administration of the chemicals, i.e. by gavage or in diet or drinking water. To extend the TTC concept to non-oral exposures, appropriate methodologies need to be developed to allow for route-to-route extrapolation. To be able to use the TTC concept for personal and household care products, including cosmetics, where dermal exposures are more important than oral exposures, the TTC concept needs further development. (VKM 2006).
It has been suggested that in the absence of data on route-specific bioavailability, an equal oral and dermal bioavailability can be assumed, and that this assumption, in the context of the TTC concept, should provide a conservative way forward (Blackburn et al. 2005, described in section 5.6).

For some cosmetic products, dermal, inhalation and oral exposures may all apply simultaneously. The TTC concept is not yet developed to deal with such multiroute exposures, and also in this context, more developed

methodology is needed. However, the TTC concept may probably be helpful in a preliminary risk assessment of an unsuspected chemical or impurity detected in a consumer product. (VKM 2006).

5.5.1 Guidance for the application of the TTC concept for cosmetic ingredients: COLIPA proposal (Kroes et al. 2007)

The European Cosmetic, Toiletry and Perfumery Association (COLIPA) has sponsored an Expert Group on the Application of the Threshold of Toxicological Concern (TTC) to the Safety Evaluation of Cosmetic Ingredients and End Products.
The possible use of the TTC concept in the safety evaluation of cosmetic ingredients was discussed at a workshop organized by COLIPA and reported by Kroes et al. (2007).

If the TTC concept should be applied to cosmetics for which human exposure occurs primarily via the topical route, comparison of the TTC values derived from oral toxicity studies with the systemic exposure to chemicals present in cosmetics would need to take into account the different route of human exposure.
Topical application and oral ingestion can result in different proportions of the applied dose entering the body as the parent compound (i.e., the bioavailability). A difference in bioavailability may arise from more extensive metabolism in the intestine and liver, compared with the skin, prior to reaching the general circulation, or slower and incomplete transfer across the skin compared with the intestinal wall, due to the physico-chemical properties of the compound. Moreover, the slower absorption after topical application results in a different shape to the plasma concentration-time curve even if the same total fraction of the dose is absorbed.

Furthermore, application of the TTC values to cosmetics has to consider various aspects of potential consumer exposure to the cosmetic end product(s).
Exposure would need to consider products that contain the relevant ingredient at the highest concentrations, and also the sum from daily cosmetics use when the ingredient is present in more than a single cosmetic end product.
In addition, the duration and frequency of human exposure to the respective cosmetic end product (e.g. rinse-off or leave-on cosmetics) has to be considered. Finally, some cosmetic products do not result in daily, but intermittent, consumer exposure. For example, direct or oxidative hair dyes are typically used at intervals of 3-8 weeks. Therefore, modifying exposure parameters should be taken into account by applying appropriate, conservative default correction values to the exposure estimation.

The Expert Group examined and discussed a number of issues related to the chemical nature and effects of ingredients and their exposure when used as cosmetics.
One issue analysed was the similarity between cosmetic ingredients and chemical classes in the Munro et al. (1996, described in section 3.2) database from which the TTC values for chemicals in food were derived. In order to address this question the chemical classes of fragrance ingredients (Bickers et al. 2003) and the substances listed in the first EC update inventory (SCCNFP 2000) were used as being representative of cosmetic ingredients and products, and these databases were compared to the chemicals from which the TTC values for chemicals in food were derived.

The other issues analysed included 1) differences in metabolism between the dermal and oral routes of application, 2) default adjustment factors for percutaneous absorption to assess the systemic exposure for topically applied cosmetics, 3) additional default adjustment factors for rinse off cosmetic products, 4) default adjustment factors for intermittent use of cosmetic products resulting in intermittent human exposure, 5) aggregate (total) exposure to the cosmetic ingredient, and 6) simultaneous dermal exposure to different cosmetic ingredients.

The analyses showed 1) that it is scientifically justified to use the TTC concept, and the database underlying the TTC values established for food chemicals, for the safety evaluation of cosmetic ingredients, and 2) that the TTC values as described earlier (Munro et al. 1996, Kroes et al. 2004, described in section 3.2 and 3.4, respectively) are appropriate for the safety evaluation of systemic exposures resulting from the use of cosmetic ingredients and products.
Proteins, heavy metals and substances with specific structural alerts of concern, which were excluded in the decision tree developed by Kroes et al. (2004), should also be excluded if the TTC concept is used for cosmetic ingredients. In addition chemicals that may have or are suspected to have pharmacological properties should also be excluded for application of the TTC concept.
The TTC values for systemic exposure to cosmetic ingredients and the use-related adjustment factors are to be regarded as provisional and could be subject to refinement when new data are developed on hazard or exposure (i.e., use pattern of cosmetic products, percutaneous absorption).

Although theoretically the TTC concept could also be applied for topical (local) effects, TTC values for local topical effects have not been developed, and at present the databases on substances producing local (topical) effects are too limited to be used as a basis for the derivation of valid TTC values.

In application of the TTC concept, appropriate exposure assessment is of prime importance. The Expert Group suggested to follow the methodology as described in the SCCNFP 'Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation' (SCCNFP 2003, Note: This version of the 'Notes of Guidance' has been replaced by a newer version (SCCP 2006) as described in the introduction to section 5.5 in this report). Depending on the use of the ingredients or products (e.g., cosmetics producing human oral exposure, cosmetics used under occlusion, cosmetics used without occlusion, rinse-off products) and the chemical characteristics of the compound under evaluation, default adjustment factors are suggested for percutaneous absorption and intermittent exposure. It was proposed that these default adjustment factors are incorporated into the exposure assessment models as described by the SCCNFP (SCCNFP 2003).
The results of the exposure assessment should then be used in the TTC decision tree shown in Figure 3. In this preliminary risk assessment all available data regarding the substance evaluated should be taken into consideration.

The following steps were suggested for application of the TTC concept to cosmetic ingredients and impurities:

1. Define product type, its intended use and related skin surface area involved

2. Define concentration of ingredient in the product
3. Estimate external exposure per day (SCCNFP 2003, US-EPA 1997)
4. Estimate skin absorption of the ingredient based on its physical and chemical characteristics
5. If a rinse-off product apply retention factor
6. Establish use pattern: e.g., daily or intermittent use, if the latter is the case apply the default factor related to the use interval
7. Calculate adjusted internal exposure per person per day (i.e., the long-term average internal dosage for a 60 kg person)
8. Where relevant, calculate total (aggregate) exposure when several cosmetic products contain this target ingredient
9. Use this average aggregate internal dosage in the TTC decision tree (Figure 3) (Note: the resulting assessment will relate to systemic but not to local effects)

The decision tree (Figure 3) comprises a series of steps, each one framed as a question, to which the answer, either 'Yes' or 'No', will carry the assessor through to the next step. The questions relate to whether the ingredient is suitable for assessment via the TTC concept, the presence of absence of structural alerts for genotoxicity, and, depending on its structure, how the level of exposure relates to the relevant human exposure threshold. For any ingredient taken through the decision tree process, one of two conclusions will be drawn: either, the substance is predicted not to be a health concern, or, further risk assessment is necessary using compound-specific toxicity data.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have concluded that the TTC approach as proposed by Kroes et al. (2007) is at present in general not applicable for risk assessment of intentionally added or formed ingredients present in cosmetic products. The same conclusion can be reached for impurities in cosmetic ingredients. In the future with validated extended databases and percutaneous absorption default factors and adequate knowledge on skin-oral route metabolism and biotransformation differences, the application for cosmetic ingredients and impurities could be further considered. The reasons for the conclusions are described in detail in section 3.7.3.4 in this report.

5.6 Consumer products, including household care products

Currently, for chemicals used in consumer products the TTC concept is not used (DG SANCO 2008). This might change in the future according to a possible broader use of the TTC concept within the REACH process (described in section 5.1.1).

The TTC concept has already been used by industry in risk assessment of ingredients in consumer products. An example is a risk assessment of isoeugenol (a fragrance ingredient in many consumer products including cosmetics and household cleaning products) by HERA (HERA 2005), an industry programme to carry out Human and Environmental Risk Assessments on ingredients of household cleaning products. (VKM 2006).

The applicability of the TTC database to ingredients in personal and household care products has been evaluated by Blackburn et al. (2005). From databases at Proctor and Gamble, repeat dose toxicity data were obtained for 248 substances used in personal care or household care products, but NOAELs could only be identified for 45 of them. Of these, 21 fall into

Cramer Class I, only 2 in Cramer Class II and 21 into Cramer Class III. The chemical structures of compounds in these products as well as their NOAELs in toxicological tests were compared with those of the chemicals in the original TTC database. The highest and mean NOAELs were similar for the two sets, but the lowest NOAELs were lower in the Munro base. According to the authors, the results showed good coverage of the product ingredient structures, and confirmed that the NOAELs for the ingredient chemicals were similar in range to the original dataset, thus supporting the use of the TTC concept also for ingredients in personal and household care consumer products.
In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have expressed that it is not possible to extrapolate the results from the Blackburn et al. (2005) study to other types of consumer products where quite different substances may be used.

In a recent publication, the application of the TTC concept to inhalation exposure for aerosol ingredients in consumer products in the context of exposure based waiving have been addressed (Carthew et al. 2009). The inhalation toxicology studies available in the public domain have been reviewed to establish a database for inhalation toxicology and to derive TTC values for effects in the respiratory tract and systemically for Cramer Class 1 and 3 chemicals, as described in section 5.9.3.
The authors suggest that these TTC values can be used as the basis for developing an exposure based waiving approach to evaluating the potential for adverse effects from exposure to ingredients in aerosol products, used by consumers. The detailed exposure evaluation for aerosol ingredients with defined use scenarios, in conjunction with an evaluation of the potential structure activity relationship for toxicity and the TTC values for inhalation exposure could be used to waive undertaking inhalation toxicology studies under REACH. They acknowledge that not all classes of chemicals are suitable for such an approach, but for chemicals with a predictable low potential toxicity, and very low levels of exposure, this approach, could reduce the amount of inhalation toxicology studies required for the implementation of the European REACH legislation.

5.7 Pharmaceuticals for human use

For human medicinal products, a TTC approach has been adopted for genotoxic impurities as described in section 4.4.1.1.

A well-established methodology exists for setting acceptable daily intake values (ADIs) and safe levels of exposure in the workplace for active pharmaceutical
ingredients (APIs) and associated intermediates when adequate toxicological data are available. These health-based limits are used to support both occupational health and quality programs in pharmaceutical research, development and manufacturing operations. The TTC concept extends the ADI methodology to address substances that have very limited or no toxicity data, but for which reasonable exposure estimates can be made, and taking the chemical structure into consideration.

In a paper by Dolan et al. (2005), ADIs were recommended, based on the TTC concept, to support pharmaceutical manufacturing quality operations, with specific application to cleaning validation and the resolution of atypical extraneous matter investigations of relatively unstudied compounds in active

pharmaceutical ingredients and finished pharmaceutical products when limited or no toxicity data is available. Recommendations were provided on ADI values that correspond to three categories of compounds; those that are likely to be carcinogenic; potent or highly toxic; or not likely to be potent, highly toxic or carcinogenic, being 1, 10 and 100 µg/person/day, respectively.

5.8 Medical devices

In the production and processing of the materials into a final product (the medical device itself), several chemical residues may be present. Currently, the TTC concept is not used for evaluation of medical devices (DG SANCO 2008).

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have expressed that the TTC concept is attractive for the use in the medical device area for chemical residues of production processes and that the TTC concept has recently become a subject of discussion for possible application in the medical device area.

5.9 The TTC concept for airborne substances

The databases used to develop the TTC values comprise experiments with oral administration of the chemicals. To extend the TTC concept to non-oral exposures such as e.g., inhalation, appropriate methodologies therefore need to be developed to allow route-to-route extrapolation. This section outlines some of the more recent analyses, suggestions and discussions in relation to extend the TTC concept to the inhalation exposure route.

5.9.1 The Concentration of No Toxicological Concern (CoNTC) concept (Drew and Frangos 2007)

Drew and Frangos (2007) developed a concept of a "Concentration of No Toxicological Concern" (CoNTC) for air toxics to be applied as a risk assessment screening tool to legitimately dismiss substances whose ground-level concentrations are predicted to be trivial.

Based on 50% of the US-FDA TTC value of 0.02 µg/kg bw/day established for carcinogens, a body weight of 70 kg and an inhalation rate of 20 m^3/day, a CoNTC of 0.03 µg/m^3 was suggested to be applied for most organic chemicals in air.
To validate the CoNTC value it was compared with established air guideline values from reputable sources all over the world. Occupational exposure limits were divided by 42 (24 hours/8 hours x 7 days/5 days x 10) where a factor of 10 was used to compensate for a greater sensitivity of the general population as compared to healthy workers. Of 1857 values taken from air guidelines from several agencies, only 4 were below the CoNTC.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR addressed the CoNTC concept, but no views have been expressed.

5.9.2 The application of the TTC concept to inhalation exposure (Escher et al. 2008)

The applicability of the TTC concept to existing chemicals relevant for REACH has been evaluated by using the RepDose database (Escher et al. 2008a,b). For the inhalation route, lower 5th percentiles were obtained for all data as well as for the individual Cramer Classes as described in section 5.1.4 and Table 9.

In order to evaluate whether inhalation thresholds are needed for industrial chemicals, preliminary inhalation TTC values were calculated in analogy with Munro et al. (1996, described in section 3.2) by multiplying the 5th percentile LOEL by 60 (average human body weight). Instead of using an assessment factor of 100, a factor of 75 was applied according to the justified adjustment factors recommended in the REACH TGD (ECHA 2008a) with a factor of 2.5 for interspecies variation, a factor of 10 for intraspecies variation, and a factor of 3 for LOEL-to-NOEL extrapolation. Furthermore, an average molecular weight of 138.2 (median molecular weight in the RepDose database) was used. All the derived inhalation TTC values were lower than those of Munro for oral exposure (Table 11).

Table 11. TTC values for inhalation compared to oral (Munro) exposure (After Escher et al. 2008b).

	Unit	Cramer Class 1	Cramer Class 2	Cramer Class 3
RepDose Inhalation	ppm	0.002	0.005	0.00093
	mmol/person/day	0.0008	0.0024	0.00021
	µg/person/day	111	332	36
Munro	µg/person/day	1796	544	88

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR noted that all derived inhalation TTC values are lower than those for oral exposure and that, according to the authors, this might be due to the inclusion of local effects (e.g. irritation) in addition to systemic effect within the RepDose database.

5.9.3 The application of the TTC concept to inhalation exposure (Carthew et al. 2009)

In a recent publication, the application of the TTC concept to inhalation exposure for aerosol ingredients in consumer products in the context of exposure based waiving has been addressed (Carthew et al. 2009), see section 5.6.

Inhalation toxicology studies available in the public domain have been reviewed to establish a database for inhalation toxicology and to derive TTC values for effects in the respiratory tract and systemically for Cramer Class 1 and 3 chemicals. More than one hundred rodent (mostly rat) studies have been conducted by industry and government agencies to evaluate the toxic potential of both gases and less volatile chemicals in aerosols. Sub-acute and sub-chronic inhalation studies were used in addition to the lifetime inhalation studies as the NOAECs from sub-acute and sub-chronic studies could be corrected to a lower NOAEC for chronic exposure using the justified adjustment factors recommended in the REACH TGD (ECHA 2008a). Inhalation studies using the rat were used because this represents the majority of the available data prepared to OECD guidelines for inhalation testing and is usually done to GLP (good laboratory practice) standards.

About twenty inhalation studies were excluded from analysis because they were on genotoxic carcinogens and use of such substances is banned under the EU Cosmetics Directive (Directive 2003/15/EC) (EC 2003). A total of 92 rat inhalation studies were reviewed using this set of criteria. In the rare instance where no NOAEC was identified, the range of endpoints was reviewed and the incidence and severity grading was used to apply an additional uncertainty factor to extrapolate from a LOAEL to a NOAEL. This was 10 for both cases in this dataset.

In order to derive a group NOAEL for use in deriving a subsequent TTC value, the 5th percentile has been used to derive, in this case, a NOAEC for local effects and also a NOAEL for systemic effects by using the default values for breathing rate and time of exposure (corrected to a 6 hour per day standard and five days a week exposure).
A designation of the Cramer class of the chemical was obtained using the Toxtree program. The 92 chemicals came roughly into 40% Class 1 and 54% Class 3 with only four chemicals in Class 2.

The TTC values for systemic effects based on oral studies were derived by applying assessment factors for inter- and intraspecies variation in toxicokinetics and toxicodynamics (10-fold, respectively), to a 5th percentile of the NOAELs derived from oral toxicology studies (Renwick et al. 2003). According to REACH, a
DNEL (derived no effect level) is derived by applying adjustment factors to the benchmark dose (5th percentile NOAEC/NOAEL) for a chemical. As the same process would be used, on the benchmark dose, the TTC value is equivalent to the group DNEL for a Cramer class derived using a 5th percentile benchmark, rather than a chemical specific NOAEL.
This overall uncertainty factor should be adjusted for differences between rat and human exposure. The REACH TGD (ECHA 2008a) specifies an assessment factor of 10 for inter-individual variations in human toxicokinetic and toxicodynamic responses. A further uncertainty factor of 2.5 is used for interspecies differences in response, for inhalation exposure, making a total of 25. Allometric scaling is not required as part of the evaluation as this has already been considered in the exposure metric for inhalation of mg/m^3 according to the REACH TGD (ECHA 2008a).
According to the authors, the overall uncertainty factor of 25 is the same as used in the derivation of the systemic TTC value from inhalation studies in the RepDose chemicals database, evaluated by the Fraunhofer Institute with reference to the poster exhibited at Eurotox 2008, Rhodes, Greece. This poster is the reference Escher et al. 2008b in this report and described in section 5.9.2. It should be noted that the point of departure used by Escher et al. (2008b) is the 5th percentile LOEL and the overall assessment factor is 75, i.e., and not a NOEL and a factor of 25 as stated by Carthew et al. (2009).

Tables 12 and 13 show the derived 5th percentile values of the NOAECs and NOAELs for local and systemic effects by Cramer class and overall for the 92 chemicals reviewed. Table 14 lists the TTC values obtained from the Cramer classes, individually and in total, for the respective local NOAECs and systemic NOAELs. The TTC values for systemic effects with Cramer Classes 1 and 3 based on inhalation data lie between the Class 1 and 3 values based on studies carried out by the oral route (Table 15). Thus, there is no huge difference between the systemic TTC values derived by data from inhalation studies compared to the TTC values derived from oral studies.

The effect of using all of the data in the inhalation database on the NOAECs, NOAELs and TTC values for local and systemic effects is also shown in Tables 12-15 for comparative purposes (Cramer 1+2+3). The spread of the Cramer Classes 1 and 3 is much lower for inhalation toxicity than for oral toxicity, being consistent with the findings of the 3-fold variation between Cramer Class 1 and 3 in the Fraunhofer RepDose work analysing inhalation toxicology studies for systemic TTC values (Escher et al. 2008b, described in section 5.9.2).

Table 12. Local NOAELs for the individual and all Cramer class chemicals derived from inhalation studies. Local effect in respiratory tract. (After Carthew et al. 2009).

Cramer class	Number in class	5th percentile for local effects NOAEC (mg/m3) for 6 hours/day	5th % NOAEL for local effects µg/g lung tissue/day [a]
1	38	1.4	54
3	50	0.47	18
1+2+3	92	0.97	38

[a] Assuming a rat lung weight of 1.4 g.

Table 13. Systemic NOAELs for the individual and all Cramer class chemicals derived from inhalation studies. Systemic effects. (After Carthew et al. 2009).

Cramer class	Number in class	5th percentile for systemic effects NOAEL (mg/kg/day)	5th % NOAEL for systemic effects µg/kg/day [a]
1	38	0.41	410
3	50	0.07	70
1+2+3	92	0.13	130

[a] Body weight used 60 kg.

Table 14. Local and systemic TTC values derived for the individual and all Cramer classes from inhalation studies. (After Carthew et al. 2009).

Cramer class	Number in class	TTC values for local effects µg/g lung tissue/day	TTC values for systemic effects µg/kg/day
1	38	2.1	16.4
3	50	0.73	2.8
1+2+3	92	1.6	5.1

Table 15. TTC values for systemic and local effects derived from oral and inhalation studies (60 kg subject). (After Carthew et al. 2009).

Cramer class	TTC values, systemic effects µg/day from inhalation exposure	TTC values, systemic effects µg/day from oral exposure	TTC values, local effects µg/day from inhalation exposure [a]
1	980	1800	1400
3	170	90	470
1+2+3	300	-	1000

[a] Using human lung weight of 650 g.

5.9.4 Evaluation of inhalation TTC values (Escher et al. 2010)

In a very recent publication, Escher et al. (2010) evaluated whether the current TTC concept can be applied to define limit values for inhalation exposure, using the database RepDose (the RepDose database is described in section 5.1.4).

Studies with chronic exposure duration (from 700 days) were prioritised. For substances which have not been investigated in a chronic study, sub-chronic (84-98 days) and then sub-acute (21-32 days) studies were used for the analysis. When more than one study in one exposure category was available the study with the lowest NOEC value was selected. For NOEC values derived from short-term studies, an extrapolation factor of 2 and 6 were

applied for sub-chronic and sub-acute studies, respectively, in accordance with the justified adjustment factors recommended in the REACH TGD (ECHA 2008a). If a NOEC could not be identified, a LOEC was extrapolated to a NOEC by applying a factor of 3 according to the REACH TGD.
Both ppm and mg/m^3 were used as dose metrics. The ppm values allow comparison of NOECs on a molar basis, whereas mg/m^3 were used for calculation of body doses and derivation of threshold values that could be directly compared to TTC values derived from the oral route.
TTC values were derived based on the 5th percentile NOEC as described by Munro et al. (1996, described in section 3.2) for the oral route. Thresholds for concentrations in air were calculated from the NOEC (ppm and in mg/m^3) of Cramer Classes 1-3 normalised to a daily exposure of 24 hours and 7 days as an exposure duration of 6 hours/day for 5 days/week is generally used in most animal studies. Subsequently, an assessment factor of 25 was used according to the REACH TGD (ECHA 2008a) with a factor of 2.5 for interspecies variation, a factor of 10 for intraspecies variation. A default value of 60 kg was used for human body weight in accordance with Munro et al. (1996) and a default value of 20 m^3 for human respiratory volume according to the REACH TGD (ECHA 2008a). In contrast to Escher et al. (2008b), the substance specific molecular weight was used in the analysis.
About 60 target organs are included in the RepDose database. To evaluate whether local and systemic threshold values have to be distinguished, thresholds were analysed for 'locally acting' and 'systemically acting' substances. Locally acting substances were defined as those showing effects predominantly at the first site of contact, i.e., before absorption has occurred. The following local target organs were defined: Eye, nose, larynx, pharynx, trachea, lung and bronchi. 'Local' as well as 'systemic' NOECs/LOECs were derived for each chemical in both subsets. NOECs were standardised using assessment factors for time and LOEC-to-NOEC extrapolation as described above.
Classification of chemicals into Cramer classes as well as putatively genotoxic or non-genotoxic was performed with the open source software Toxtree.

A total of 203 industrial chemicals were analysed to derive TTC values for inhalation exposure (Table 16).
Most of the chemicals (68%) fell into Cramer Class 3 (toxic) with 29% in Cramer Class 1 (non-toxic) and only 3% in Cramer Class 2 (moderately toxic). This distribution is similar to the grouping obtained by Munro et al. (1996, described in section 3.2).
The Cramer classes of the RepDose database data set were compared with the limit values of the GHS classification (Globally Harmonised System of Classification and Labeling of Chemicals) for repeated dose toxicity: toxic 15 ppm, harmful > 15 and 80 ppm, low toxicity > 80 ppm. These limit values were obtained by dividing the LOECs originally provided by GHS by 3 to extrapolate from LOECs to NOECs and thus, to allow comparison with the NOECs from the inhalation studies. The majority of the chemicals was also classified as toxic according to the GHS criteria. A comparison of the distribution of NOEC valuess in Cramer Classes 3 and 1 with the classification according to the GHS showed that 47% of all substances can be considered as toxic in Cramer Class 1 and 31% as harmful or of low toxicity in Cramer Class 3. Based on this comparison, the authors concluded that the Cramer and GHS classifications disagree on the grouping of chemicals for many chemicals of the RepDose database data set. It should be noted that this conclusion is based on an analysis of systemic effects following inhalation.

The NOECs observed in the 203 inhalation studies covered a wide range from 0.001 to 100,000 ppm. About 19% of the chemicals had very low NOECs (below 0.1 ppm) and about 15% had very high NOECs (above 100 ppm). The majority of compounds in Cramer Class 1 had NOECs between 0.1 and 10,000 ppm and Cramer Class 3 covered NOECs between 0.001 and 100,000 ppm. The wide spread of NOECs in both classes thus result in a remarkable overlap between the two classes of low-toxicity and toxic substances. Although the NOECs for Cramer Classes 1 and 3 overlap to some extent, the 5th percentile NOECs for the the three Cramer classes each differed by a factor of about ten, thus resulting in three clearly discriminated TTC values of 0.0015, 0.0002 and 0.000022 ppm for Cramer Class 1, 2 and 3, respectively, (Table 16). As only 7 chemicals were available for deriving the TTC value for Cramer Class 2, the authors considered this TTC value to be unreliable. The corresponding TTC values as internal doses were 71, 10 and 4 µg/person/day for Cramer Class 1, 2 and 3, respectively. These TTC values for inhalation exposure are all considerably lower than the TTC values of 1800, 540 and 90 µg/person/day for Cramer Class 1, 2 and 3, respectively, derived by Munro et al. (1996, described in section 3.2) for the oral route.

Table 16. TTC values for inhalation exposure (After Escher et al. 2010).

	Cramer class	N (%)	NOEC (ppm) 5th percentiles	TTC (ppm)	TTC (mg/m^3)	TTC (µg/person/day)
All chemicals N = 203	1 2 3	58 (29%) 7 (3%) 138 (68%)	0.21 0.028 0.0031	0.0015 0.0002 0.000022	0.0036 0.00048 0.00018	71 10 4
Chemicals with local targets N = 102	1 2 3	26 (25%) 6 (6%) 70 (69%)	0.021 0.028 0.0033	0.00015 0.0002 0.000024	0.00061 0.00048 0.00019	12 10 4
Chemicals with only systemic targets N = 97	1 2 3	29 (30%) 1 67 (69%)	0.34 - 0.006	0.0024 - 0.000043	0.0048 - 0.00039	95 - 8
All chemicals with systemic targets N = 199	1 2 3	55 (28%) 7 (3%) 137 (69%)	0.25 0.52 0.006	0.0018 0.0037 0.000043	0.0048 0.011 0.00032	95 214 6

It was further evaluated whether the low TTC values derived for inhalation exposure are due to a special sensitivity of the respiratory tract to local effects because of e.g., irritating properties or differences in metabolism/bioactivation in the lung compared to the liver, which may lead to higher toxicity in the lung.

For Cramer Class 1 and to a lesser extent for Cramer Class 3, classical systemic targets such as liver or kidney were affected less frequently at study LOEC compared to local target organs such as nose or lung, thus indicating that local effects in the respiratory tract play a major role in determining the LOEC, which may trigger the observed low TTC values.

The 203 chemicals were sub-grouped into chemicals with local targets (102 substances) and chemicals with only systemic targets (97 substances); four chemicals which did not cause any effects in the corresponding studies were excluded from this analysis (Table 16).

For the subset of chemicals with local targets, most of the chemicals (69%) fell into Cramer Class 3 with 26% in Cramer Class 1 and only 6% in Cramer Class 2; this is similar to the classification for the group of all 203 chemicals. For Cramer Class 1, the TTC value for chemicals with local targets (0.00015 ppm) was lower compared to the TTC value for all chemicals with systemic targets (0.0018 ppm). For Cramer Class 3, the 'local' TTC value (0.000024 ppm) did not differ significantly from the 'systemic' TTC value (0.000043 ppm). The authors have mentioned that the data sets used to derive these TTC values are relatively small, so that the 5th percentile NOECs were determined by only a fes substances of high toxicity (1 substance in Cramer Class 1 and 3-4 substances in Cramer Class 3). When comparing the distribution of NOECs characterised by the geometric mean or median value of the data set, both values were lower for 'local' toxicity than for 'systemic' toxicity for Cramer Class 1, whereas this trend was less evident for Cramer Class 3.
For the subset of chemicals with only systemic targets, the systemic NOEC corresponded to the study NOEC. For Cramer Class 1, the 'local' TTC value (0.00015 ppm) was lower compared to the 'systemic only' TTC value (0.000043 ppm); also the median and geometric means were higher compared to the values for the 'local' TTC. For Cramer Class 3, the 'local' TTC value (0.000024 ppm) did not differ significantly from the 'systemic only' TTC value (0.000043 ppm); also the median and geometric means were in the same range so that a trend towards a lower TTC value for 'local' toxicity is less evident.
Overall, this analysis indicated that the lower thresholds derived for inhalation exposure compared to the oral route are due to the respiratory tract being a sensitive target organ in the inhalation studies.

It was also analysed whether there were any particular structural classes that accounted for the large differences between systemic and local NOECs in the inhalation studies. For 19 chemicals, the ratio between systemic and local NOECs was higher than 9. The most frequently occurring structural class was that of carboxylic esters (7 substances, 37%). Other structural classes identified included α,β unsaturated carbonyls, aliphatic ethers, halogenides, diisocyanates, diketones and secondary amines.

By excluding organophosphates (OPs) and chemicals with structural alerts for genotoxicity it was further evaluated whether the general inhalation TTC values for Cramer Classes 1 and 3 are sufficiently conservative or would be shifted to higher values (Table 17). Fourteen OPs belong to Cramer Class 3 were identified and 67 genotoxic compounds with 5 in each of Cramer Class 1 and 2, and 57 in Cramer Class 3.
For Cramer Class 3, the TTC value (0.000024 ppm) derived after exclusion of OPs or genotoxic compounds was similar to the general TTC value (0.000022 ppm), thus indicating that the general TTC value was sufficiently conservative for all the chemicals in the data set.
For Cramer Class 1, the TTC value (0.0036 ppm) derived after exclusion of the five genotoxic compounds was about a factor of two higher than the general TTC value (0.0015 ppm). This indicates, according to the authors, that reactive compounds with structural alerts for genotoxicity should be excluded from Cramer Class 1.

Table 17. TTC values for inhalation exposure (After Escher et al. 2010).

	Cramer class	N (%)	NOEC (ppm) 5th percentiles	TTC (ppm)	TTC (mg/m³)	TTC (µg/ person/day)
All chemicals N = 203	1 3	58 (29%) 138 (68%)	0.21 0.0031	0.0015 0.000022	0.0036 0.00018	71 4
OPs excluded N = 189	1 3	58 (31%) 124 (66%)	0.21 0.0033	0.0015 0.000024	0.0036 0.00018	71 4
Genotox excluded N = 136	1 3	53 (39%) 81 (60%)	0.50 0.0033	0.0036 0.000024	0.0089 0.00018	180 4

In conclusion, the authors proposed a general inhalation TTC value of 0.0036 ppm (0.0089 mg/m^3 / 180 µg/person/day) for Cramer Class 1, excluding genotoxic compounds, and a general inhalation TTC value of 0.000024 ppm (0.00018 mg/m^3 / 4 µg/person/day) for Cramer Class 3 (Table 17). A TTC value for Cramer Class 2 was not proposed, as the Cramer decision tree grouped only few substances into this class. Slightly higher inhalation TTC values could be derived for workers by adjusting the exposure to 8 hours a day and 5 days per week.
The general TTC values expressed in ppm are recommended for single compounds with known molecular weight and high vapour pressure.
The general TTC values expressed in mg/m^3 are recommended for aerosols and for mixtures, provided the structures of the (major) components are known.
The general TTC values expressed in µg/person/day might be applicable in cases where consumers encounter exposures of short duration (less than 8 hours/day) to aerosols.

5.10 The TTC concept for endpoint specific areas

5.10.1 Allergic contact dermatitis (Safford 2008)

In the present form, the TTC concept refers to systemic toxicity, in which allergic contact dermatitis is not considered as an endpoint.

Recently a proposal was published to use the TTC concept for dermal sensitisation as well (Safford 2008).

The incidence of sensitizers in the world of chemicals was estimated using the ELINCS (European List of Notified Chemical Substances) data set, and a distribution for sensitisation potency was established using a compilation of Local Lymph Node Assay (LLNA) data. From the analysis of these data sets a 'Dermal
Sensitisation Threshold' (DST) was established below which, according to the author, there is no appreciable risk of sensitisation, even for an untested ingredient.

The approach further built on the recently published method of the Quantitative Risk Assessment (QRA) for fragrances (Api et al. 2008). Based on the results of the LLNA data sensitisation thresholds for humans (NESILs) were derived and these were subsequently converted to an acceptable exposure level using a number of assessment factors. The DST was then determined for each product type as a 95th percentile in the

distribution. This implies that using this DST there is a 5% probability that an untested chemical would give an undue risk. The author indicated that the choice of the percentile is certainly a matter of risk management and can be debated. He also noted that the DST will be protective for induction, but not for the elicitation of sensitisation.

In their recent opinion (DG SANCO 2008), the EU Scientific Committees SCCP/SCHER/SCENIHR have expressed that the proposal to use the TTC concept for dermal sensitisation based on the dermal sensitisation QRA method may, after refinement and validation, in the future be applicable for risk assessment of new substances to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitization QRA model and validation must be performed employing a broad range of different chemicals and data from substantial clinical investigations. In addition scientific consensus must be obtained, especially concerning the choice of safety factors in the model. As a consequence, the concerns stated by the SCCP for the dermal sensitization QRA also refer to the TTC concept for allergic contact dermatitis and make, at this time, the TTC concept not applicable for this endpoint.

5.10.2 Exposure-triggered reproductive toxicity testing (Bernauer et al. 2008)

In certain regulatory approaches, an additional assessment factor (of up to 10-fold) is used for teratogenicity and it might be argued that teratogens should be considered as a separate class when applying the TTC concept. The ILSI Europe Expert Group (Kroes et al. 2004, Barlow 2005) decided that the application of an additional assessment factor for teratogenicity is not needed in the application of the TTC concept and that a separate TTC class for teratogenic effects would not be necessary as described in section 3.4.

Bernauer et al. (2008) have, however, decided to apply an additional assessment factor for reproductive toxicity when proposing cut-off values of human exposure for reproductive toxicity endpoints as described in the following section.

As mentioned in the introduction to chapter 5.1, exemption from conducting individual toxicity tests (waiving) is possible in cases where exposure is to be neglected. Attempts have been made to establish cut-off criteria between “non-relevant” and “relevant” (detrimental) exposure based on external exposure concentrations and the TTC concept.

The paper by Bernauer et al. (2008) make a proposal and describe a strategy how to define the currently insufficiently described terms “relevant/significant” exposure, see Section 5.1.5.

In the context of exposure-based waiving, the authors would propose to use an “endpoint-specific TTC”for the toxicological endpoint under consideration. For the establishment of endpoint-specific TTC values existing data on NOAEL or NOAEC values for specific endpoints can be used to determine their empirical distribution and to define a cut-off value. This has been demonstrated in the paper for effects on fertility and developmental toxicity.

As a database for fertility and developmental toxicity the authors selected chemical substances assessed under the EU existing chemicals program. NOAELs/NOAECs were obtained for 91 substances. The data were analysed

separately for the endpoints fertility and developmental toxicity and also for the route of exposure (oral and inhalation).
For the oral route, 58 NOAELs for fertility and 62 NOAELs for developmental toxicity were obtained for the oral route. NOAELs for fertility ranged from 1.5 to 17,260 mg/kg bw/day and for developmental toxicity from 1.1 to 2430 mg/kg bw/day. For the inhalation route, 24 NOAECs were obtained for fertility and 24 NOAECs for developmental toxicity. NOAECs for fertility ranged from 0.1 to 36,900 mg/m^3 and for developmental toxicity from 0.05 to 24,500 mg/m^3.
The authors did some simulation to explore the distribution, which, however, was difficult because of the limited number of data. Instead, the authors would propose to use the lowest value represented in the data set as an appropriate cut-off rather than a predefined percentile.

In the next step, an appropriate assessment factor was selected. The standard default factors of 10 for inter- and intraspecies variability, respectively, were selected as these cannot be reduced in the absence of data. An extra factor of 10 was proposed because of the uncertainty inherent in the small database (91 substances) and because the effects are serious health effects (in accordance with the procedure to establish DNEL(s) as described in Annex I 1.4.1 letter (b) of the REACH regulation (EC 2006b). And also in line with the proposal by other authors that an additional factor of 10 should be applied in deriving TTC values (Cheeseman et al. 1999, described in section 3.3; Frawley 1967 and Rulis 1986, both described in section 3.1). The resulting overall assessment factor for the oral route is 1000. Hence, the proposed TTC values for oral uptake are 1.5 µg/kg bw/day for fertility and 1.0 µg/kg bw/day for developmental toxicity (see Table 18).

For the NOAECs (inhalation), no assessment factor has been applied for interspecies extrapolation taking into account the experimental conditions (6 hours
exposure/day for 5 days/week in the rat versus 24 hours exposure/day for 7 days/week in humans), the minute volume (0.35 m^3/day in rats versus 15.2 m^3/day in humans) and the body weight (0.2 kg for rats versus 75 kg for humans). Hence, the overall assessment safety factor in deriving TTC values for inhalation exposure is 100 (factor 10 for interindividual variability and factor 10 as an additional factor). The resulting TTC values for uptake via inhalation are 1 µg/m^3 (fertility) and 0.5 µg/m^3 (developmental toxicity) (see Table 18).

In order to further elucidate whether the additional assessment factor of 10 is appropriate, the authors used data from oral prenatal toxicity studies in the rat for 507 pharmaceuticals submitted to the German Federal Institute for Drugs and
Medical Devices between 1985 and 2000. NOAELs for embryo- and foetotoxicity ranged from 0.0001 to 9600 mg/kg bw/day; the 5th percentile was 0.1 mg/kg bw/day. A default factor of 100 (10 for inter- and intraspecies variability, respectively) was applied with the resulting TTC value being 1 µg/kg/day.

Table 18. Cut-off values of human exposure for reproductive toxicity endpoints derived from the database contained in the EU risk assessment programme of existing chemicals. (After Bernauer et al. 2008).

	TTC value oral (µg/kg bw/day)[a]	TTC value inhalation (µg/m³) [b]
Fertility	1.5	1.0
Developmental toxicity	1.0	0.5

[a] Derived from the lowest oral values for the respective endpoints represented in the database (existing chemicals EU) and divided by an assessment factor of 1000.
[b] Derived from the lowest inhalative values for the respective endpoints represented in the database (existing chemicals EU). Corrected for experimental conditions, minute volume and body weight and divided by an assessment factor of 100.

The TTC value derived from the much larger database of pharmaceuticals (compared to industrial chemicals) is identical to the one obtained from industrial chemicals by applying an assessment factor of 1000. The authors concluded therefore, that uncertainty of the data and severity of effect would very well justify an extra assessment factor of 10 in the case of industrial chemicals.

The resulting oral endpoint-specific TTC values are higher than the general TTC value of 1.5 µg/day (0.02 µg/kg) proposed by Kroes et al. (2000, 2004, described in section 3.4) and lower than Cramer Class I and II chemicals (Cramer et al. 1978).

In the context of reproductive toxicity, continuous or repeated exposure and not single exposure is considered. However, the distinction between single and repeated exposure should carefully be made for developmental toxicity as in selected cases in animal studies even single dose exposure applied in the sensitive window did lead to developmental effects (van Raaij et al. 2003).

The authors noted that the Nordic Council of Ministers (NCM 2005, described in section 5.1.2) came to the conclusion, that application of the TTC concept (at or above 100 tons per year) within REACH would be premature and raised some concerns, for example that the TTC concept has not been evaluated for the diverse group of industrial chemicals and for different routes of exposure. Acknowledging that the derivation of the TTC values has limitations, the authors considered such uncertainties by introducing an additional factor of 10 to the standard default factors.

The authors also noted that Kroes et al. (2004, described in section 3.4) came to the conclusion that the usual 100-fold assessment factor would be sufficient to cover teratogenic effects. However, the authors decided to apply an additional assessment factor considering inter alia that reproductive toxicity are to be regarded as serious health effects (in accordance with REACH) and noted that this is in line with the caution presented in a publication of Bokkers and Slob (2007) who came to the conclusion that applying an interspecies default assessment factor of 10 could result in human exposure scenarios that are insufficiently protective.

5.10.3 TTC for prenatal developmental toxicity (van Ravenzwaay et al. 2010)

Van Ravenzwaay et al. (2010) acknowledged that the TTC concept has now been widely accepted, is used in a regulatory context and has been extended from chemicals occurring in food to other exposure scenarios. The authors noted, however, that most of the reported work refers to chronic toxicity and

carcinogenicity and that only a limited number of reports deal with endpoint specific TTC values. As the authors expected that most of the toxicological testing under the REACH legislation will be in the area of reproductive toxicity, they considered that the development of TTC values for reproductive toxicity could contribute significantly to a reduction in animal testing. They therefore have evaluated the possibility of developing a TTC value for prenatal developmental toxicity by analyzing the NOAEL/LOAEL values of 93 different OECD TG 414 test guideline studies in rats performed in their own laboratories (BASF) during the last two decades.

From the 93 BASF studies performed according to OECD TG 414, the NOAEL and the LOAEL for maternal (92 studies) and developmental toxicity (93 studies) were identified and taken for analysis. The cumulative distribution function of the NOAEL/LOAEL values was plotted and the median values as well as the 5^{th}, 10^{th}, 90^{th} and 95^{th} percentiles were determined. Histograms were plotted for the molecular mass and the octanol-water coefficient for 88 BASF studies. In cases that no toxic effects were noted in the maternal animals or when no developmental toxicity was observed at the highest dose tested, the LOAEL value for statistical analysis was set at the NOAEL value of the study in question. This was considered to be a conservative approach. An additional analysis of the data were based on the data reported by Kroes et al. (2004) and a total of 18 studies with NOAEL values reported to be performed in rats following oral administration, and calculations were performed in the same way.

The 5^{th} percentile of the NOAEL distributions were calculated to be 4 mg/kg bw/day for maternal and 5 mg/kg bw/day for developmental toxicity. Adding the data for developmental toxicity provided by Kroes et al. (2004), a joint evaluation of 111 individual NOAEL values resulted in a 5^{th} percentile of 4 mg/kg bw/day. By application of an assessment factor of 500 to account for a possible underrepresentation of chemical classes on the 5^{th} percentile, a TTC value for developmental toxicity of 8 μg/kg bw/day was calculated based on the combined data, and of 8 μg/kg bw/day for maternal toxicity based solely on the BASF data. According to the authors, this value may serve as a guidance within the REACH context whether to perform an animal experiment or to rely on a TTC value if estimated exposure is sufficiently low.

6 The TTC concept: Benefits, limitations, and uncertainties

The enhanced structure based tiered Threshold of Toxicological Concern (TTC) concept as outlined by Kroes et al. (2004) and Barlow (2005) as described in section 3.4, building on the original publications by Munro et al. (1996, described in section 3.2) and Cheesemann et al. (1999, described in section 3.3) and amended by Felter et al. (2009, described in section 5.2.4) refers to the establishment of human exposure threshold values for groups of chemicals, below which there would be no appreciable risk to human health.

The concept that safe levels of exposure can be identified for individual chemicals is already widely accepted in the current practice of setting acceptable or tolerable daily intakes (ADIs, TDIs) 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.

6.1 The TTC concept: Benefits

The major benefits of using the TTC concept are twofold:

- It can be used to assess the safety of oral human exposure via food or the environment to low levels of a chemical of known structure even in the absence of toxicological data, and
- it can be used for priority setting for those compounds that exceed the exposure threshold and therefore need a more extensive risk assessment, i.e. by allocating resources for toxicity testing and development of analytical methods to fulfil data needs for exposure assessments.

Thus, application of the TTC concept:

- Avoids unnecessary extensive toxicity testing and safety evaluations when human exposures are below such a threshold, and
- focuses the resources on those substances which are estimated to have greater potential to pose risks to human health.

The decision tree described by Kroes et al. (2004) (see Figure 3 in section 3.4 in this report) with several later modifications for specific assessment purposes could 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 classification of chemicals as ‘‘of concern’’ based solely on hazard identification without taking into account potential exposure and considerations of predicted ***in vivo*** potency, could lead to an unnecessarily large number of compounds requiring extensive hazard characterization using

in vivo studies in experimental animals. The TTC concept provides an approach 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.

Knowledge about the structure and inherent properties of the chemical is needed for the tiered TTC approach using the Cramer et al. (1978) structural classes (described in section 3.2). This requires expertise in organic chemistry, biochemistry, SARs, (Q)SARs etc. General knowledge on the metabolism of chemicals, as well as knowledge of whether the substance or its metabolites are endogenous in the human body, is also important aspects in using the Cramer et al. (1978) structural classes.

In order to promote the potential use of the TTC concept within REACH, the European Chemicals Bureau has commissioned the development of a computer software program (toxTree) to encode the Cramer classification scheme. ToxTree (Version 2.1.0) is available as a free download upon registration from the Ex-ECB website at http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/.

6.2 The TTC concept: Limitations

The main limitations of the TTC concept are:

- Until now, only oral exposure has been succesfully covered
- A number of compounds and groups of compounds are not covered
- Certain toxicological endpoints are not covered
- Exposure assessments have not yet been discussed in great detail and no international consensus has been achieved
- The TTC concept is developed for single chemicals / structural groups, not for chemical mixtures.

The databases used to develop the general TTC concept (Munro et al. 1996, described in section 3.2; Cheeseman et al. 1999, described in section 3.3) are exclusively based on studies using oral administration of the chemicals.
One example to extend the TTC concept to cover inhalation exposure is the analyses performed with the RepDose database developed at the Fraunhofer Institute for Toxicology and Experimental Medicine (described in section 5.9.2 and 5.9.4). Another example is the proposed application of the TTC concept to inhalation exposure for aerosol ingredients in consumer products (described in section 5.9.3).
Application of the TTC concept for dermal exposure has been discussed in the context of cosmetic ingredients (described in section 5.5.1).
Although serious efforts are currently done in order to extend the TTC concept to cover also the inhalation and dermal exposure routes, appropriate methodologies still need to be developed.

As described in section 3.4 and outlined in Figure 3, certain chemicals and structural groups are excluded from the general TTC concept, including (heavy) metals, polyhalogenated dibenzo-p-dioxins, polyhalogenated dibenzofurans, polyhalogenated biphenyls, proteins, high molecular weight compounds (polymers), steroids, and chemicals with structural alerts for

genotoxicity such as aflatoxin-like, azoxy- and ***N***-nitroso-compounds. Also compounds known to accumulate in the body should be excluded.

As the databases used to develop the general TTC concept are exclusively based on systemic effects after repeated oral administration of the chemicals, acute toxicity and local effects such as irritation and sensitisation are not covered. Also endpoints like allergic reactions, hypersensitivity, and intolerance are not covered. A proposal for use of the TTC concept for dermal sensitisation has been published by Safford (2008, described in section 5.10.1).

Exposure data are essential in any risk assessment procedure. Whereas the chemical and toxicological background behind the development of the TTC concept has been adequately documented and agreed upon by several authors and expert groups, the accompanying exposure assessments have not yet been discussed in great detail and no international consensus has been achieved. In case exposure assessments are lacking or are of insufficient scientific quality, worst case scenarios are regularly applied. As the TTC concept introduces an additional level of uncertainty in the hazard assessment, the need for sound exposure data becomes even more imminent.

It should be recognised that the TTC concept can only be applied for single substances with known chemical structure as the concept has not been developed to cover mixtures of chemicals. Thus, combined exposures in terms of exposure to multiple chemicals with the same mode of action are not covered by the general TTC concept. Future developments that potentially might strengthen the usefulness of the TTC concept would be to explore the application of the TTC concept to mixtures of chemicals.

6.3 The TTC concept: Uncertainties

Similarly to the traditional risk assessment approach, the TTC approach fully relies on the underlying database, i.e., the assessment cannot be better than the available data allow.

6.3.1 The database behind the TTC concept

The database behind the current TTC concept is based on the majority of the published oral toxicity studies having an appropriate quality for use in risk assessments. And it covers a wide range of different groups of chemicals. Reviews of these data were used to establish threshold levels of exposure, related to the chemical structure, which would be without appreciable risk, despite the absence of toxicity data on the compound being considered.

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. Therefore, in the traditional hazard assessment of a chemical, an uncertainty (assessment/extrapolation) factor is applied on the most sensitive, relevant NOAEL derived from the toxicological studies, in order to cover the inherent variability among species and individuals as well as uncertainties in the testing.
The TTC values for the three Cramer et al. (1978) structural Classes I, II, and III of 90, 540 and 1800 µg/person per day, respectively, were based on

the 5th percentile NOEL for each structural class converted to a human exposure threshold by applying the conventional default uncertainty factor of 100 (as described in section 3.2). This means that the possibility exists that the TTC value for a given structural class would not provide optimal protection for 5% of compounds allocated to that structural class, i.e. the uncertainty factor could be less than 100.

The general TTC value of 1.5 µg/person/day (0.025 µg/kg bw/day), used by US-FDA in the Threshold of Regulation (TR) policy (described in section 4.1.1), and by the JECFA in the assessment of flavouring agents that do not show structural alerts for genotoxicity and carcinogenicity (described in section 4.2.1), and the later introduced TTC value of 0.15 µg/person/day (0.0025 µg/kg bw/day) for carcinogenic and genotoxic compounds (Kroes et al. 2004, described in section 3.4), was designed to protect against the toxicity of most chemicals, including those of unknown toxicity should they turn out to be carcinogenic. However, five groups of compounds were identified having a significant fraction of their members that may still be of concern at an intake of 0.15 µg/person/day (0.0025 µg/kg bw/day). Three of these groups are genotoxic; the aflatoxin-like, azoxy- and ***N***-nitroso-compounds, while two groups were non-genotoxic; the 2,3,7,8-dibenzo-***p***-dioxin (TCDD) and its analogues, and the steroids. Such compounds with these structural alerts for high carcinogenic potency require compound-specific toxicity data and should be excluded from any TTC approach. A TTC value of 0.15 µg/person/day could be used for all other substances with structural alerts for genotoxicity.

The TTC values are derived from databases that cover systemic toxicity after sub-chronic and chronic oral administration. The TTC values therefore do not take account for effects after inhalation and dermal contact, and do not cover acute toxicity and local effects such as irritation and sensitisation.

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 TTC concept should not be used for such substances. Compounds with extremely large half-lives that show very large species differences in bioaccumulation, such as TCDD and analogues were not in the original database of Munro et al. (1996, described in section 3.2) and are also excluded from consideration by the TTC concept. In addition, although the data available do not permit the establishment of a clear causal link between endocrine active chemicals and adverse effects in humans, the issue of potential low-dose effects of hormone-like chemicals remains unresolved, and therefore such chemicals should not be evaluated using the TTC concept. Finally, the traditional threshold approach has never been applied to sensitisation, and a NOEL based on allergy ever been established. Thus, more data are necessary to determine threshold doses for allergens.

It is also important to be aware that the substance specific data from which the NOAEL values have been derived and used for the generation of the three structural classes mainly are from the 1960-ties to the 1980-ties (Munro et al. 1996) and thus an updating of this documentation could very well have an influence on the NOAEL distributions as presented in Figure 1 (section 3.2) in this report.
It should be noted that the DG SANCO Expert Group concluded that further research is needed in the development and validation of the current toxicity

databases particularly in the areas where an insufficient number of representative chemicals is included (DG SANCO 2008).

The DG SANCO Expert Group noted that there is a major overlap of NOELs and LOELs between Cramer Class I, II and III and expressed that it would be desirable to achieve a better separation of the classes and consequently the TTCs based on them. The Cramer classification was developed in 1978 on theoretical considerations and might be improved by analysis of outliers in the Classes and by incorporating recent experience on QSAR and modes of action into the decision tree. (DG SANCO 2008).

Future developments that potentially might strengthen the usefulness of the TTC concept would be to explore the application of the TTC concept to mixtures of chemicals. This should not involve additional challenges than those already pertinent in traditional risk assessments of chemical mixtures. As an example, assessment of combined exposure is already performed by the JECFA and the EFSA for groups of structurally related flavouring agents. JECFA and EFSA perform an evaluation of the combined exposure in the (unlikely) event that all of the substances in the group (or a sub-group) are simultaneously consumed on a daily basis. In the case that the estimated combined daily per capita human intake does not exceed the TTC value for the structural classes in question, it is concluded that there would be no safety concerns associated with the combined intake.

6.3.2 The exposure assessment

It has to be stressed, that the databases used to develop the TTC concept comprise experiments with oral administration of the chemicals (i.e., by gavage or in diet or drinking water) and thus, the threshold values currently used in the TTC concept are related to oral exposures, expressed in μg per person per day.

Humans are exposed to chemicals via ingestion, inhalation or dermal contact, and it is the dose at the target organ that is critical. In most cases, however, the dose at the target organ is difficult to determine and therefore generally is substituted with the internal or the external exposure. Therefore, the TTC value 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.

In order to apply the TTC concept in risk assessment, information on human exposure is thus of crucial importance and it is important to ensure that exposure estimates are as complete and accurate as possible, or that they are built on adequate conservatism to account for possible underestimates.

Use of the TTC concept in future risk assessments of chemicals therefore would require estimates of reliable exposures, which may not be easily available. However, an estimate of exposure is also necessary in traditional risk assessments and thus, is not a particular feature related to the TTC concept. As in many traditional assessments, an initial screening of exposure would use conservative methods, such as for example the Budget Method used for food additives with the assumptions described in the report of the SCOOP Task 4.2 (EC 1998b) or EUSES for industrial chemicals. The same approach could be applied for a first screening of potential exposure when the TTC

concept is used. In both cases an exceedence of the ADI/TDI or the relevant TTC exposure, respectively, would flag a health concern and trigger either a refinement of the exposure assessment or call for further toxicological data.

The TTC concept is relevant for substances where data are lacking or where data are very sparse. In general, for substances with very few data, also very little is known about the specific exposure levels. In such cases the risk assessor has to rely on read-across to other substances with comparable physico-chemical properties and comparable exposure situations, or the risk assessor has to use exposure models. Exposure models are however, often conservative and overestimate exposure and furthermore, many of these models have been developed to predict exposure at a level comparable with occupational exposure levels, which in general are orders of magnitudes higher than exposure levels used in the various TTC approaches. Thus, there is a lack of models that, on a valid basis, can predict low levels of human exposure, and an increased use of the TTC concept is therefore closely associated to further development of models that can predict exposure at low levels.

For a specific substance, it is important to identify all exposure pathways to estimate the total exposure. For example, a substance present in a cosmetic product may also be used in a food package or in a consumer product as well as occurring as a contaminant in drinking water. Moreover, cosmetic products are usually applied on the skin, but ingestion of products applied on the lips or inhalation of a substance released from products in spray form may also contribute substantially to the total exposure.

In order to extend the TTC concept to non-oral exposures, appropriate methodologies need to be developed to allow route-to-route extrapolation. It is also necessary to develop methodology to assess combined multi-route or multi-pathway exposures. Advances in exposure modelling should also cover the need for assessments of such aggregate exposures. Combined exposures in terms of exposure to multiple chemicals with the same mode of action should be given attention. Chemicals which are assumed to accumulate in the body should ***a priori*** be excluded from the TTC concept. Since particular groups in the population may use different amounts of specific foods and consumer products, exposure data may need to be sufficiently detailed to enable these groups to be examined separately, for example by age, gender or ethnicity.

7 Potential applications of the TTC concept by the Danish Environmental Protection Agency and the Danish Veterinary and Food Administration

Proposals for potential applications of the TTC concept in the resort areas of the Danish Environmental Protection Agency (MST) and the Danish Veterinary and Food Administration (FVST) are outlined in the subsequent sections.

It should be noted that the benefits, limitations and uncertainties described in chapter 6 are of utmost importance when considering the potential applications of the TTC concept suggested in this chapter.

7.1 Priority settings

For the MST and the FVST, a benefit of using the TTC concept would be to avoid unnecessary use of resources for risk assessment of chemicals for which human exposure is below the relevant TTC values. This would allow the available, limited resources of time, cost and expertise to be used for evaluation of those substances with the potential to pose higher risks to human health. Thus, the TTC concept may be useful in setting priorities for allocating resources for risk assessments, toxicological testing, development of analytical methodologies, and more refined exposure assessments.

The TTC concept can be used to identify those substances for which exposure estimates exceed the relevant TTC value and therefore may require further information for risk assessment. Such information may be more toxicological testing, depending on their structure and the degree to which they exceeded the TTC value.

The TTC concept may also be applied to identify substances for which available analytical methods needs improvement in order to allow for accurate measurements at concentrations relevant to their particular structural class TTC value.

Substances, for which exposure estimates are uncertain, but close to the relevant TTC value, may require more refined estimates of exposure. Therefore, the TTC concept may help in setting priorities for exposure assessments of chemicals in food and the environment.

The TTC concept can most easily be applied where exposure is defined on beforehand, i.e., in areas where standard exposure is used, e.g., in relation to the intake of drinking water and soil. In other, less well defined exposure situations it may be necessary to make predictions of exposure based on the

physico-chemical properties of the chemical and the different parameters that may influence the magnitude of exposure. In these situations, further use of the TTC concept will be dependant of development of valid tools for exposure modelling at low exposure levels comparable to the TTC values.

7.2 Applications specific for the Danish Environmental Protection Agency

The TTC concept is considered useful for the Danish Environmental Protection Agency (MST) in situations where very low exposure to chemicals are to be assessed as well as for priority settings as described in the previous section.

It is therefore suggested that the MST proceeds in implementing the TTC concept and use it in specific cases. Initially, in considering the approach provided by Kroes et al. (2004, described in section 3.4) it should be identified in which areas the MST can use the concept. In addition, it should be discussed which modifications may be necessary. In particular it is relevant to consider which TTC values should be used because a stringent subdivision into the three Cramer et al. (1978, described in section 3.2) classes can be difficult to perform without a more detailed knowledge about structure activity relationships and metabolic routes. It therefore might be most appropriate to use the more conservative TTC values from Kroes et al. (2004), because the higher values, Class II and I, requires a more detailed evaluation of the structure and metabolism of the compound. It is also recommended to involve (Q)SAR, as the (Q)SAR models can guide in the evaluation of potential critical effects, and thereby aid in placing the compound in the decision tree, as well as models for predicting the metabolism of a chemical substance, e.g., the model 'METEOR'.

Application of the TTC concept could be considered by the MST within the following areas:

- industrial chemicals, REACH
- drinking water contaminants and materials intended for contact with drinking water
- non-persistent contaminants in soil
- ambient air pollutants (at present only for systemic effects)
- personal care products, including cosmetics (some reservations, see section 7.2.5)
- consumer products, including household care products
- genotoxic impurities

However, as described in section 6.2, there are a number of limitations for the application of the TTC concept in general in terms of the applicability of the database, the excluded classes of chemicals, and the fact that only systemic effects after oral exposure are covered.

For application of the TTC concept for most of the above-mentioned areas, further development and adjustment of the TTC concept is therefore needed. One important aspect is that the TTC concept has to be extended to include the inhalation and dermal exposure routes besides the oral exposure route. A promising attempt to extend the TTC concept to cover inhalation exposure is the analyses performed with the RepDose database developed at the

Fraunhofer Institute for Toxicology and Experimental Medicine (described in section 5.9.2 and 5.9.4). Application of the TTC concept for dermal exposure has been discussed in the context of cosmetic ingredients (described in section 5.5.1). These attempts to extend the TTC concept to cover inhalation and dermal exposure are considered too limited for regulatory purposes for the time being but may, after refinement and validation, in the future be applicable for assessment of substances in areas where these exposure routes are relevant.
Another important aspect is that endpoints such as acute toxicity, irritation and sensitisation are not covered by the TTC concept. A proposal for use of the TTC concept for dermal sensitisation has been published by Safford (2008, described in section 5.10.1). This proposal is considered too limited for regulatory purposes for the time being but may, after refinement and validation, in the future be applicable for assessment of chemicals prior to incorporation into consumer products, including cosmetics.

7.2.1 Industrial chemicals, REACH

As described in section 5.1.1 the use of the TTC concept has been discussed in connection with REACH.

The generic TTC concept illustrated in Figure 5 (in section 5.1.1) could be applied for the chemical safety assessment for data poor substances at low tonnage levels triggering limited information on systemic effects from repeated dose toxicity and/or reproductive toxicity studies as well as for substances for which data do not allow for identification of a N(L)OAEL from which a DNEL value can be derived.

Another area for application of the TTC concept is in relation to exposure based waiving. In the guidance document for the implementation of REACH (REACH TGD) (ECHA 2008a) information on criteria for waiving certain studies is provided, including for the use of the TTC concept, where the documentation of an exposure level below a certain TTC level would make further testing superfluous.
It should be noted, however, that there is no reference to any threshold values in the REACH TGD. A proposal for exposure-triggered toxicity testing by using the TTC concept has been published by Bernauer et al. (2008, described in section 5.1.5) and demonstrated for effects on fertility and developmental toxicity (described in section 5.10.2). However, further development and adjustment is still needed.

In addition, exposure models for low level exposure comparable to the general TTC levels have to be developed before a wider use of this concept could be used in REACH.

It should also be noted that, when using the TTC concept under REACH no information on classification and labelling of a chemical or on its potency for a certain effect is provided.

7.2.2 Drinking water contaminants and materials intended for contact with drinking water

For contaminants in drinking water as well as from materials intended for contact with drinking water, the most relevant exposure route for most contaminants is the oral route.

In general, a health-based quality criterion for a specific chemical in drinking water is calculated from a tolerable daily intake and a standardised intake of drinking water (MST 2006).

The TTC concept seems to be a useful tool for the assessment of data poor chemical substances in drinking water and in materials intended for contact with drinking water as standardised exposure estimates are used for the intake of drinking water.
The TTC values proposed by Kroes et al. (2004, described in section 3.4), as amended by Felter at al. (2009, described in section 5.2.4) could be considered as a relevant starting point provided that systemic effects are considered as being the critical effect(s) for the chemicals in question.

7.2.3 Non-persistent contaminants in soil

For contaminants in soil, the most relevant exposure route for most contaminants is the oral and the dermal route.

In general, a health-based quality criterion for a specific chemical in soil is calculated from a tolerable daily intake and a standardised exposure to soil (MST 2006).

The TTC concept could be considered for the assessment of data poor non-persistent chemical substances in soil in relation to oral intake as standardised exposure estimates are used for the intake of soil.
The TTC values proposed by Kroes et al. (2004, described in section 3.4), as amended by Felter at al. (2009, described in section 5.2.4) could be considered as a relevant starting point provided that systemic effects are considered as being the critical effect(s) for the chemical in question.
For an assessment in relation to dermal contact, the TTC concept needs further development and adjustment.

7.2.4 Ambient air pollutants

For ambient air pollutants, the most relevant exposure route for most contaminants is the inhalation route.

In general, a health-based quality criterion for a specific chemical in ambient air is calculated from a tolerable daily intake or a tolerable concentration and a standardised inhalation exposure (MST 2006).

The TTC concept could be considered for the assessment of data poor chemical substances in ambient air as standardised exposure estimates are used for the inhalation exposure.
However, for an assessment in relation to ambient air, where inhalation exposure is relevant compared with oral exposure, the TTC concept needs further development and adjustment. At present, it seems that the TTC concept might be useful for assessments in relation to systemic effects whereas methodologies for assessing local effects needs to be much further developed if possible at all.

7.2.5 Personal care products, including cosmetics

A TTC approach has not yet been applied in the safety evaluations of cosmetic ingredients at the EU level. The TTC concept is mentioned in the SCCP's 'Notes of Guidance' (SCCP 2006, described in section 5.5) and the

possible use of the TTC concept for the safety evaluation of cosmetic ingredients has been discussed in a COLIPA Expert Group and reported by Kroes et al. (2007, described in section 5.5.1).

For assessments of data poor chemicals in personal care products, including cosmetics, where dermal exposures are much more relevant than oral exposure, the TTC concept needs further development and adjustment. It has been suggested that, in the absence of data on route-specific bioavailability, an equal oral and dermal bioavailability can be assumed, and that this assumption, in the context of the TTC concept, should provide a conservative way forward (Blackburn et al. 2005, described in section 5.6). However, this assumption needs further evaluation before an application for regulatory purposes can be considered.

7.2.6 Consumer products, including household care products

For some consumer products, dermal, inhalation and oral exposures may all apply simultaneously. The TTC concept is not yet developed to deal with such multi-route exposures, but may probably be helpful in a preliminary risk assessment of an unsuspected chemical or impurity detected in a consumer product.

For assessments of data poor chemicals in consumer products, including household care products, where inhalation and dermal exposures often are more important than oral exposures, the TTC concept needs further development and adjustment. At present, it seems that the TTC concept might be useful for assessments in relation to systemic effects whereas methodologies for assessing local effects needs to be much further developed if possible at all.

7.2.7 Genotoxic impurities

The European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) has released a "Guideline on the Limits of Genotoxic Impurities", which recommends the application of a TTC concept for defining acceptable limits of genotoxic impurities present in drug substances (described in section 4.4.1.1).

The regulatory experiences with the TTC concept since coming into force of the CHMP guideline in January 2007 show that this concept can be used as a pragmatic and very helpful tool for the regulation of genotoxic impurities in new drug substances (DG SANCO 2008).

Such an approach could also be considered for genotoxic impurities in e.g., pesticides and biocides as well as in other chemical use categories.

7.3 Applications specific for the Danish Food and Veterinary Administration (FVST)

7.3.1 Risk assessment of substances occurring in low concentrations in food

The TTC concept is already used within the EU for flavourings agents in food as described in section 4.2.2. Its use should be considered by the FVST for wider application for the assessment of:

- substances migrating from food contact materials
- veterinary drug residues
- non-persistent contaminants found in animal feed, its raw materials, or additives
- residues of pesticide metabolites
- some mycotoxins
- naturally occurring toxicants in food plants
- food contaminants originating from the environment or processing
- substances used as processing aids at low concentrations in a very limited number of food items.

Thus, the TTC concept could be a valuable tool as a preliminary, and sometimes only, step in a risk assessment of chemicals identified to be present at low concentrations in food, for which toxicity data are lacking, but for which exposure assessments can be undertaken.

Use of the TTC approach provided by Kroes et al. (2004, described in section 3.4), as amended by Felter et al. (2009, described in section 5.2.4) is advocated.

The TTC concept is not designed to replace conventional approaches to risk characterisation for established and well-studied chemicals, such as pesticides and food additives. However, the TTC concept may be used to evaluate small amounts of unintended impurities and/or breakdown products of such compounds. Also, the separate step for organophosphate pesticides in the TTC decision tree (Figure 3 in section 3.4 in this report) is not intended to replace the normal regulatory assessments and controls for organophosphates used as pesticides, but can be used to determine whether there is any concern should a non-approved or unregulated organophosphate be detected in food as a contaminant.

7.3.2 Disinfectants used in the food industry

The DTU National Food Institute advises the FVST on the food safety resulting from the use of disinfectants by the food producing industry for decontamination of surfaces in production plants that may potentially come into contact with food. The DTU National Food Institute has decided to use the TTC concept as provided by Kroes et al. (2004, described in section 3.4), in those cases where toxicological data are scarce but the contamination of the food and the subsequent consumer exposure is estimated to be very low.

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