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Threshold of Toxicological Concern (TTC)

Literature review and applicability

TemaNord 2005:559

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Nordic Environmental Co-operation

Environmental co-operation is aimed at contributing to the improvement of the environment and forestall problems in the Nordic countries as well as on the international scene. The co-operation is conducted by the Nordic Committee of Senior Officials for Environmental Affairs. The co-operation endeavours to advance joint aims for Action Plans and joint projects, exchange of information and assistance, e.g. to Eastern Europe, through the Nordic Environmental Finance Corporation (NEFCO).

Nordic co-operation

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Content

Preface

A Nordic project on information strategies (NOIS) was started in order to produce quick and early input to the REACH Implementation Project on information strategies (RIP 3.3).

The REACH proposal contains testing requirements that are mainly dependent on production volumes. To get a practical system, additional guidance is needed on how to obtain the necessary information (by using intelligent sequences of testing or information gathering), how to use different kinds of information, how to make decisions based on different types of data (e.g., when to stop testing and/or take action). The guidance could be produced by elaboration of testing or information strategies (including the use of QSAR, grouping, read across, *in vitro* and *in vivo* data, and release/exposure related information), with data generation in a stepwise approach, where, in principle, a higher level would correspond to more relevant, certain and accurate data. Thus, the implementation of the REACH proposal requires development of Integrated Information Strategies (IIS), that will assist in designing testing programs that give useful information while still being animal- and resource saving.

It has sometimes been proposed that a Threshold of Toxicological Concern (TTC) concept could be used as an alternative tool, where estimated general effect values are used as substitute for substance specific information for comparison with the available exposure information. Based on this comparison, it has been stated that decisions can be taken on whether waving further testing is appropriate or not. In this project, the aim is to analyse how different TTC-like concepts have been used and assess their potential usability in REACH.

This report has been prepared under a consultant contract with the Nordic Chemicals Group of the Nordic Council of Ministers. The report has been written by; Alicja Andersson, Agneta Falk-Filipsson, Jörgen Henriksson, Bengt Melsäter, Helena Parkman, and Maria Wallén (The Swedish Chemicals Inspectorate).

The work has been guided by a Nordic Steering group, with the following members from the Nordic authorities: Bert-Ove Lund and Ivar Lundbergh representing the Swedish Chemicals Inspectorate (KemI), Sweden; Gunnlaug Einarsdóttir representing the Environment & Food Agency (UST), Iceland; Jaana Heiskanen representing the Finnish Environment Institute (SYKE), Finland; Marko Kuittinen and Kirsi Sihvonen representing the National Product Control Agency for Welfare and Health (STTV), Finland; Lotte Kau Anderson and Henrik Tyle representing the Danish Environmental Protection Agency (MST), Denmark; Toralf Kaland and Vibeke Sømnes representing the Norwegian Pollution Control

Authority (SFT), Norway. The steering group, as well as other colleagues at Nordic authorities responsible for chemical safety have provided valuable information and input to the work.

Abbreviations and acronyms

ALV	Adjusted Logarithmic Value
B	Bioaccumulating
BCF	BioConcentration Factor
CHMP	Committee for Medical Products for Human use
CPDB	Carcinogenic Potency Database
CPMP	Committee for Proprietary Medical Products (see CHMP)
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
EC	European Commission
EC_{50}	Effect Concentration
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EFSA	European Food Safety Authority
EMEA	European Medicines Agency
EP	Equilibrium Partitioning
ETNC	Exposure Threshold of No Concern
EU	European Union
FAO	Food and Agricultural Organisation in the U.S
FEMA	Flavor and Extract Manufacturers Association
GEV	Generic Exposure Values
GLEV	Generic Lowest Effect Values
ILSI	International Life Sciences Institute
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC_{50}	Lethal Concentration
LD_{50}	Lethal Dose
LOEC	Lowest Observed Effect Concentration
MALV	Mean Adjusted Logarithmic Value
MIC	Minimum Inhibitory Concentration
MOA	Mode Of Action
NCI	National Cancer Institute
NFC	Natural Flavour Complexes
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
P	Persistent
PEC	Predicted ExposureEnvironmental Concentration

PNEC	Predicted No Effect Concentration
ppb	parts per billion
ppm	parts per million
QSAR	Quantitative Structure Activity Relationship
REACH	Registration, Evaluation, Authorisation of CHemicals
T	Toxic
TD_{50}	Tumour Dose
TGD	Technical Guidance Document
TOR	Threshold of Regulation
tpa	tonnes per year
TTC	Threshold of Toxicological Concern
U.S. EPA	U.S. Environmental Protection Agency
U.S. FD&C	U.S. Food, Drug and Cosmetic
U.S. FDA	U.S. Food and Drug Administration
WHO	World Health Organisation

Summary

Threshold of Toxicological Concern (TTC) based on the analysis of the toxicological and/or structural data of a broad range of different chemicals has been developed as substitute for substance specific information in the regulation of food contact articles and flavouring substances.

Use of the TTC concept has recently been proposed as a tool in performing risk assessment of industrial chemicals within REACH. However, at time present it is concluded that it is too premature to use the concept due to limitations and uncertainties in the derivation of TTCs as well as to the fact that the TTC concept has not yet been evaluated for the diverse group of industrial chemicals. In addition, it is doubtful whether it is possible to obtain a sufficient level of protection in risk assessment of industrial chemicals by the use of the TTC concept.

Human Health Risk Assessment

There exists two principal approaches in the use of general toxicological thresholds: a general TTC based on carcinogenicity data, and a TTC based on structural information in combination with toxicological data of chemicals ("the decision tree approach") for non-carcinogenic endpoints.

Currently, the TTC value used by the U.S. FDA (U.S. Food and Drug Administration) is 1.5 μg/person/day for food contact articles. Concerning flavouring substances the TTC values for regulatory purposes adopted by EFSA (European Food and Safety Authority) and JECFA (Joint FAO/WHO Expert Committee on Food Additives) are 90, 540 and 1800 μg/person/day. In addition to these values JECFA uses the value of 1.5 μg per person and day.

An approach suggested by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) intended for use in the regulation of industrial chemicals within the new chemical strategy REACH (Registration, Evaluation, Authorisation of Chemicals) comprises the use of generic threshold values based on hazard categories. For risk assessment of consumers the Generic Lowest Effect Values (GLEVs) are based on the EU classification limit for repeated dose toxicity, while for occupational exposure Generic Exposure Values (GEVs) are derived from occupational exposure limits (OELs). Both GLEVs and GEVs are adjusted with assessment factors.

Environmental Risk Assessment

There is presently no use of the TTC concept as regards regulatory environmental assessments. However, two different approaches that can be seen as environmental TTC, i.e. the "action-limit" and the "$ETNC_{aquatic}$"

(ETNC; Exposure Threshold of No Concern) have been found in the literature.

The action-limit is part of a proposed step-wise, tiered procedure for the environmental risk assessment of pharmaceuticals (for human use) by European Medicines Agency (EMEA). The proposed action-limit has been questioned by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) since drugs with lower effect concentrations were found. In addition, the focus on acute toxicity was questioned, as chronic toxicity was considered more relevant for pharmaceuticals.

A different TTC-approach was applied deriving an environmental ETNC for the pelagic freshwater compartment, i.e. $ETNC_{aquatic}$. This approach was based on existing toxicological databases and substance hazard assessments for organisms in the freshwater environment, and a categorisation of chemicals into four different modes of action. The stratified data was fitted to a lognormal distribution from which a fifth percentile, with a 50% confidence interval, was determined. This value was then divided by an assessment factor, ranging from 1 to 1000 depending on the data, to obtain the $ETNC_{aquatic}$.

As the environmental TTC approaches are developed only for direct effects on the pelagic freshwater ecosystem, no effects due to bioaccumulation, or accumulation in other compartments are taken into consideration. Additionally, the concept does not cover metals, other inorganic compounds, and ionisable organic compounds. The use of non-testing information, as compared to experimental data, implies a higher risk of not considering the toxicity of degradation product(s)/metabolite(s), which may prove important if they are more toxic than the parent compound.

N.B. The present document does not include any policy laid down in the Nordic Chemicals Group but is intended to serve as a basis for future policy-making in the Nordic countries.

Sammanfattning

Generella toxikologiska tröskelvärden (TTC; Threshold of Toxicological Concern), baserade på analyser av toxikologiska och strukturella data för ett stort antal olika kemikalier används för närvarande som ersättning för substansspecifik information för reglering av kemikalier i varor som kommer i kontakt med föda och aromämnen i livsmedel. Användning av generella toxikologiska tröskelvärden har dessutom föreslagits som verktyg i riskbedömningen av industrikemikalier inom REACH.

Slutsatsen av arbetet med denna rapport är att TTC för närvarande inte bör användas vid riskbedömning av industrikemikalier på grund av (1) begränsningar och osäkerheter vid framtagandet av TTC, (2) att TTC ännu inte utvärderats för den mångfacetterade gruppen industrikemikalier, samt (3) att det kan ifrågasättas om en tillräckligt hög skyddsnivå kan uppnås då generella toxikologiska tröskelvärden ersätter substansspecifik information.

Hälsoriskbedömning

Idag används TTC-värden framtagna på två principiellt olika sätt inom hälsoriskbedömning. Ett generellt värde, 1,5 µg/person/dag, är baserat på data från en cancerdatabas och används av U.S. FDA (Amerikanska Livs- och Läkemedelsmyndigheten) för reglering av varor som kommer i kontakt med livsmedel. Andra värden, 90, 540 och 1800 µg/person/dag, grundas på strukturinformation i kombination med toxikologiska data från andra effekter än cancer och används av EFSA (Europeiska Livsmedelsmyndigheten) och JECFA (Joint FAO/WHO Expert Committee on Food Additives) för riskbedömning av aromämnen i livsmedel. JECFA använder även värdet 1,5 µg/person/dag.

ECETOCs (European Centre for Ecotoxicology and Toxicology of Chemicals) förslag att använda generella tröskelvärden i riskbedömningen av industrikemikalier inom REACH baseras på farokategorier. När det gäller riskbedömning vid konsumentexponering utgår GLEV (Generic Lowest Effect Value) från EUs klassificeringsgräns för toxicitet vid upprepad exponering, och när det gäller riskbedömning vid yrkesmässig exponering utgår GEV (Generic Exposure Values) från yrkeshygieniska gränsvärden. Både GLEVs och GEVs justeras med bedömningsfaktorer.

Miljöriskbedömning

Det finns för närvarande ingen regulatorisk användning av TTC när det gäller miljöriskbedömning. Däremot finns två olika tillvägagångssätt, ”action-limit” och ”$ETNC_{aquatic}$”, (ETNC; Exposure Threshold of No Concern) beskrivna i litteraturen.

Action-limit är en del av ett av stegvist tillvägagångssätt för miljöriskbedömning av läkemedel föreslaget av Europeiska Läkemedelsmyndigheten (EMEA). Den föreslagna gränsen har ifrågasatts av CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment) eftersom man fann läkemedel som kan ge effekter vid lägre koncentrationer. Dessutom ifrågasattes att fokus låg på akuttoxicitet då kronisk toxicitet ansågs mer relevant när det gäller riskbedömning av läkemedel i miljön.

När det gäller att ta fram ett ETNC för den fria vattenmassan i sötvattenmiljö tillämpades ett annat tillvägagångssätt. ETNC baseras på existerande toxikologiska databaser och farobedömningar för organismer i sötvatten, samt en kategorisering av kemikalier i fyra olika verkningssätt (modes of action). Stratifierade data anpassades till en logaritmisk normalfördelning från vilken en femte percentil med ett 50% konfidensintervall fastställdes. För att erhålla $ETNC_{aquatic}$ dividerades detta värde med en faktor på mellan 1-1000 beroende på data.

När det gäller effekter på miljön är TTC endast utvecklad för direkta effekter på den fria vattenmassan i sötvattenmiljö. Detta innebär att inga effekter som uppkommer efter bioackumulering eller ansamling i andra delar av miljön tas med i beräkningen. Dessutom täcker konceptet inte metaller och andra oorganiska föreningar, samt joniserbara organiska föreningar. Användningen av icke test information jämfört med experimentella data i en riskbedömning kan innebära en högre risk på grund av att nedbrytningsprodukt(er)/metabolit(er) kan vara mer toxiska än modersubstansen.

1. Introduction

The establishment of TTC is based on the analysis of the toxicological and/or structural data of a broad range of different chemicals. The TTCs might be used as substitute for substance specific information in situations where there are limited or no information on toxicity of the compound and where the human exposure is so low that undertaking toxicity studies is considered not warranted, due to animal welfare considerations and of the costs incurred in manpower and laboratory resources.

In this context, it should be recognized that the concept that exposure thresholds, below which no (adverse) effects can be expected, can be identified for individual chemicals is widely accepted and used by different regulatory bodies.

Initially the TTC concept was derived for low dose exposure to food contact articles and flavouring substances, i.e. substances with specific and limited use, and at present, there are some different TTC values available for substances in food at low levels.

Use of the Threshold of Toxicological Concern (TTC) concept has been proposed by European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 2004) as an alternative tool in performing risk assessment of chemicals within the legislation as suggested in REACH (Registration, Evaluation, Authorization of CHemicals) (EC, 2003a; b).

1.1 Aim

This project was set up in order to increase the knowledge of the concept and of the present application by different regulatory bodies and furthermore to consider its applicability in relation to risk assessment, especially within REACH. The result of the project should serve as a basis for the Nordic countries to help in their decision-making with respect to the possible use of TTC and its derivates in risk assessment of chemicals within REACH (EC, 2003a; 2003b).

2. Human health

This chapter is a literature review of the different TTC approaches and applications. We have chosen not to take a definite position at this stage, instead it is the different authors' own view that is presented. Comparisons and discussions are found in the Discussion.

2.1 Literature review

There exists two principal approaches in the thresholds developed for food contact articles and flavouring substances to date: First, a general TTC mainly based on carcinogenicity data, and second a TTC based on structural information compared with toxicological data of chemicals ("the decision tree approach") for non-carcinogenic endpoints. The different TTC values that are based on these approaches are illustrated and summarized in Table 1.

In 1958 the Food Additives Amendment to the Federal Food, Drug and Cosmetic Act defined that contact material and their components that might migrate unintentionally into food was included in the definition of a food additive (U.S. FD&C Act, 1958). This in combination with the development of more sensitive and discriminating analytical methods implied that there was a need for a policy at the United States Food and Drug Administrations (U.S. FDA) how to handle low dose exposure. While still protecting the public health in the event that the substance turn out to be a carcinogen the U.S. FDA wanted to be able to waive requested tests in certain cases, and to be consistent in this waiving procedure. During several years discussions went on concerning how to establish the level of a "Threshold of regulation".

The general TTC was based on TD_{50} values from a carcinogenic potency database derived by Gold *et al*, (1984). A distribution plot of the chronic dose rates was set up, and extrapolation to a distribution of 10^{-6} risk to develop cancer was performed (Rulis, 1986, 1989; Flamm, 1987). The estimated value of 0.5 ppb, corresponding to 1.5 μg/person/day was implemented by the U.S. FDA in 1995 as the "Threshold of Regulation for food contact material" (U.S. FDA, 1995).

The TTC based on structural information was suggested by Munro (1996) and is based on a decision tree developed in 1978 by Cramer *et al*. Munro compiled a database of 612 organic substances including the structure and the distribution of No Observed Effect Levels (NOEL) for chronic, sub chronic, reproductive toxicity for a wide variety of organic chemicals. Carcinogenic and mutagenic endpoints were not considered.

Human exposure thresholds of 1800, 540 and 90 μg/person/day were proposed for class I, II and III, respectively, using the 5th percentile of the lowest NOEL for each substance and a safety factor of 100. European Food Safety Authority (EFSA) has adopted these values in the year 2000 for the regulation of flavourings substances used in food (EC, 2000).

The TTC principle has also been adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its evaluations of flavouring substances (JECFA, 1995). Both the general TTC and the TTC based on structural information are in use. Information on the metabolism is also included in the application of the TTC. The values adopted by JECFA are 90, 540, and 1800 μg per person and day (Cramer *et al*., 1978; Munro *et al*., 1996). In addition, the value of 1.5 μg per person and day, estimated by the U.S. FDA, is used.

In a tiered approach, methods were established for extending the U.S. FDA concept for food contact articles by using structure-activity relationships, genotoxicity, and short-term toxicity data (Cheeseman *et al*., 1999). The thresholds of concern suggested are: 1) general threshold of 1.5 μg/person/day, 2) a threshold of 15 μg/person/day for chemicals without structural alerts for carcinogenicity or with negative mutagenicity test, and 3) a threshold of 45 μg/person/day for chemicals without structural alerts for carcinogenicity or with negative mutagenicity test (Ames test) and with an appropriate acute toxicity test with LD_{50} >1000 mg/kg bodyweight.

Recently, an approach intended for the regulation of industrial chemicals within REACH was suggested by ECETOC (2004). It comprises the use of generic threshold values based on hazard categories. Inclusion in hazard categories depends on the substance's specific classification (or no classification) according to Directive 67/548/EC (EC, 1967). For consumers the Generic Lowest Effect Values (GLEVs) are based on EU criteria for the classification of the substance for repeated dose toxicity (R48 "danger of serious damage to health by prolonged exposure") after adjustment with an assessment factor. Further, for occupational exposure Generic Exposure Values (GEVs) are derived from Occupational Exposure Limits (OELs) after adjustment with an assessment factor.

The Committee for Medicinal Products for Human use (CHMP) at European Medicines Agency (EMEA), have in a draft "Guideline on the limits of genotoxic substances" proposed a general framework and practical approaches on how to deal with genotoxic impurities in drug substances and excipients (EMEA/CPMP, 2004). The application of a TTC value in the assessment is proposed. It should be noted that the TTC for genotoxic carcinogens reflects the threshold for an acceptable increased risk and not the threshold below which no (adverse) effects can be expected. This EMEA draft has been on an external review and the result from this review is not yet available.

Table 1 Comparison of different TTC approaches and values (oral exposure only).

	U.S. FDA	JECFA[1]			EFSA			Cheeseman			ECETOC		
Terminology	Threshold of Regulation	Threshold of Toxicological Concern, and Threshold of regulation			Threshold of Toxicological Concern			Threshold of Regulation			General lowest effect value (GLEV)[2]		
Intended use[3]	Food contact articles	Flavouring substances			Flavouring substances			Food contact articles			All chemicals		
Approach	Threshold of regulation approach and *de minimis*	Decision tree approach (and Threshold of regulation approach and *de minimis*)			Decision tree approach			Tiered approach			Hazard category approach		
Based on	carcinogens; TD_{50}	non-carcinogens; NOEL carcinogens; TD_{50}			non-carcinogens; NOEL			carcinogens; TD_{50} non-carcinogens; NOEL			EU criteria for classific.of repeated dose toxicity (R48)		
Background references	Rulis, 1989	Munro *et al*., 1996; Rulis, 1989			Munro *et al*., 1996			Cheeseman, 1999			ECETOC, 2004		
Thresholds groups		Class			Class			Level					
		III	II	I	III	II	I	3	2	1	high	medium	low
Values, µg/person/day	1.5	90	540	1800	90	540	1800	1.5	15	45	150	1500	15000

1 In addition, for food contact articles the value of 1.5 µg per person and day, estimated by U.S. FDA, is used.

2 The GLEVs obtained from the hazard category approach is intended for consumers. For occupational exposure the corresponding threshold is GEV. As GEV is derived for inhalation exposure and not for oral exposure it is not included for comparison in the Table.

3 With exceptions mentioned in the text

2.1.1 Cited references

Frawley (1967) was the first to present an analysis to establish a generic threshold value (threshold of regulation) or ranges values with the aim to reduce extensive toxicity studies and safety evaluations, and to address, within the available capacity, those substances for which the potential or actual intake is substantial. This was based on distribution of chronic (2-years) rodent studies on 220 substances. 19 of the compounds were toxic below 10 ppm. It was shown that all 19 were heavy metals and pesticides. In addition, 39 of the 40 substances, which were toxic below 100 ppm, were heavy metals and pesticides. The only substance in the "all other compounds" category which was toxic below 100 ppm was acrylamide. Subdividing the substances in the subgroups "heavy metals and pesticides" and "all other compounds" is discussed, and exclusion of the former group is suggested from this approach. Applying the conventional 100-fold factor to the "no-effect" levels of the "all other compounds" would imply that the substances are safe for man at a dietary concentration of 100 ppb.

Gold *et al.* (1984) presented the Carcinogenic Potency Database (CPDB), which in year 1984 included data on approximately 3000 long-term, chronic animal experiments with about 770 different chemicals. Sources, from where data have been collected, are the NCI/NTP (National Cancer Institute/National Toxicology Program) bioassays and bioassays published in the open literature. For each experiment in the database a TD_{50} value was calculated. The definition given by Gold and co-workers of a TD_{50} value is: "For a given target site(s), if there are no tumours in control animals, then TD_{50} is that chronic dose rate in mg/kg bw/day which would induce tumours in half the test animals at the end of a standard lifespan for the species". A general approach was to calculate a TD_{50} for each category of neoplasm, benign and malignant, which an author evaluated as treatment related, regardless of the statistical or biological basis for the evaluation.

Since 1984 the CPDB has been updated and in 2004 it included analyses of experimental design for 6008 experiments, including species, strain, route of administration, dose and protocol on in total 1451 chemicals (**Gold *et al.*, 2004**).

The concept of a "threshold of regulation" (TOR) for food additives is addressed by **Rulis (1986, 1989)**. Some thoughts from the U.S. FDA, and some recommendations for making further progress of the concept are discussed. The approach is based upon the premises 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 that can be said to fall below some "threshold

of regulation". Substances in question would not necessarily need to undergo the rigors of the premarket safety evaluation requirements. Known carcinogens would be subject to more formal risk assessment.

Data on 343 oral carcinogens from animal studies compiled by Gold *et al.* (1984) in the Carcinogenic Potency Database was used as an illustrative example in the paper. Distributions of TD_{50} values from different databases were also used. Ongoing work by the time of the publication indicated that use of the relationship between acute toxicity and carcinogen potency was of dubious practical value (Rulis, 1986).

The potency of the carcinogenic substances was defined as the slope of a straight line connecting the point representing TD_{50} with the point representing zero risk and zero dose. When the potencies were grouped into ranges and plotted as a probabilistic distribution they formed a normal distribution. The distribution was transformed into an exposure distribution at a constant assumed risk of $1x10^{-6}$ per lifetime (this risk level has been chosen because it is the upper bound level of risk identified by the U.S. FDA as *de minimis* for the purpose of regulating the carcinogen methylene chloride as a coffee decaffeinater (**U.S. FDA, 1985**)). The resulting curve 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, by using the probability curves developed it was predicted that should a substance permitted under a "threshold of regulation" unknowingly be a carcinogen, it would have 60% risk of presenting greater than a $1x10^{-6}$ per lifetime at the 5 ppb level of exposure; one at that time suggested level for use as a threshold of regulation.

It is concluded by the authors that it appears to exist an adequate scientific basis of data and information on which to construct a "threshold of regulation" policy relating to food-contact substances, and that once the U.S. FDA is able to support a given migration level or range of levels as acceptable for a "threshold of regulation" it will be a better position to make judgements on consistent basis.

In 1993 the U.S. 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. The proposal was in response to a number of comments from representatives of the food packaging and processing industries suggesting that the U.S. FDA should establish a threshold of regulation policy ("threshold of regulation") whereby those substances used in food-contact articles that result in minimal migration into food would be exempt from regulation as food additives. The threshold for exemption from the regulation was 0.5 ppb or less for substances used in food-contact articles.

Although a number of comments expressed the opinion that the 0.5 ppb threshold is more conservative and restrictive than is necessary to

adequately protect the public health, no data were submitted that would justify the U.S. FDA establishing a threshold of regulatory concern at a dietary concentration level higher than 0.5 ppb. Based on its analysis of the available evidence, the U.S. FDA concludes that this evidence does not support a threshold significantly higher than 0.5 ppb, especially where the substance being considered for an exemption has not been the subject of any toxicological testing. Further, concerning carcinogenic effects, because such effects typically occur at lower dietary concentrations than those at which non carcinogenic toxic effects occur, a 0.5 ppb threshold would ensure that substances that pass under this level pose negligible safety concerns from non carcinogenic toxic effects as well. Therefore, this final rule establishes 0.5 ppb as the threshold of regulatory concern for substances intended for use in food-contact articles (**U.S. FDA, 1995**).

Due to the adoption of the Threshold of Regulation concept by the U.S. FDA (1995) in the context of food contact materials, the European Scientific Committee for Food concluded that the concept behind the threshold of regulation policy, that is to say, the proposition that there is a level of exposure to non-carcinogenic chemicals in the diet below which, even in the absence of toxicity data, there is reasonable assurance that no adverse effects would occur in man, is a sound one (**EC, 1998b**). Further, the Committee concluded that it was necessary to conduct an up-to-date-review to cover important endpoints of concern, which may give rise to effects at low doses, like immunotoxic, endocrinologic, neurotoxic and developmentally toxic events. In the Committee's view the mathematical methods used for extrapolating from experimental animal studies are not sufficiently scientifically well-founded to persuade it that they provide a valid basis for estimating the true risk from very low doses. Nevertheless, the committee recognises that the linearised multistage model used incorporates a number of conservative assumptions, and probably provides protection against a large number of genotoxic carcinogens. Finally, the committee recommended that resources should be given to appraise new developments in risk assessment methodology and, should suitable and robust methods emerge, work towards an appropriate framework of guidelines on how such methods might be applied to the various areas of the Committee's activities.

By the use of a decision tree **Cramer *et al.* (1978)** seeks unambiguously to classify every structurally defined organic or metallo-organic chemical by criteria that are based largely on structure or on widely known facts of biochemistry or physiological chemistry. The information on the substances was taken from handbooks such as the Merck Index. The test substances were: 247 substances reported to cause cancer, and a number of food additives, drugs, industrial chemicals and pesticides (all listed in the report). The description of the decision tree includes 33 steps on organic chemistry, biochemistry and food chemistry to classify chemi-

cals into three classes (I = low priority for testing, II = medium priority for testing, III = high priority for testing). The thresholds for oral toxicity are: Class I limit > 50 mg/kg/day; Class II limits 5 mg/kg/day - 50 mg/kg/day; Class III limit < 5 mg/kg/day.

Munro (1990) presented a paper where he analyzed the database of approximately 350 substances compiled by Gold *et al. (*1984) to develop a human exposure threshold to be applied to substances used for food packaging materials for which no presumption of safety can be made because of a complete lack of data on metabolism and toxicity.

Based on the Rulis (1986) methodology a threshold of regulation of up to 1 ppb for indirect additives depending on assumptions regarding food intake was proposed. It was also suggested that structure/activity relationships and *in vitro* short-term genotoxicity may rule out that a chemical is a genotoxic carcinogen.

Munro *et al. (*1996) made a compilation of a database of 612 organic substances including the structure and the distribution of No Observed Effect Levels (NOEL) for chronic, sub chronic, and reproductive toxicity for a wide variety of organic chemicals. Carcinogenic and mutagenic endpoints were not considered. The chemicals were divided into three classes with respect to their chemical structure according to the principals established by Cramer *et al. (*1978). The analysis of the database indicated that the distributions of NOEL differ significantly for the three structural classes of chemicals.

Human exposure threshold was proposed for the three classes using the 5th percentile of the lowest NOEL for each substance (3, 0.91 and 0.15 mg/kg bw/day, respectively, for class I, II and III). Using a safety factor of 100 this corresponds to human exposure thresholds of 1800, 540 and 90 μg/person/day, respectively. 24 of 448 class III chemicals (pesticides, drugs and industrial chemicals) fell below the 5th percentile (8 of 448 substances fell below the first percentile), i.e. the toxicity for some substances was underestimated.

A few years later **Munro *et al.*, (1998; 1999)** reviewed the principles and procedures for the safety evaluation of flavouring substances. The majority of flavouring substances are members of groups of substances with common metabolic pathways, and typically, individual members of such a group display a similar toxicity profile. Intake of flavouring substances is usually low, and in the majority of cases, below the human exposure threshold. They also developed criteria in form of critical questions that always should be asked in the evaluation of flavouring substances. The safety evaluation procedure was shown to provide a scientifically based practical method of integrating data on intake, structure-activity relationship, metabolism and toxicity to evaluate flavouring substances. In a comparison of sensitivity of various endpoints analysis of 31 organophosphates, with cholineesterase-inhibition as an endpoint, derived a TTC (using the 5th percentile of the lowest NOEL for each

substance and a safety factor of 100) of 18 µg/person/day, i.e. below the lowest limit of the three classes I, II and III (1800, 540 and 90 µg/person/day).

Cheeseman *et al.* (1999) extended the TTC concept as adopted by the U.S. FDA (1995) further by establishing a tiered approach to threshold of regulation by using structure-activity relationships, genotoxicity, and short-term toxicity data. Cheeseman also discussed the possibility to raise the threshold of 0.5 ppb used by the U.S. FDA to a dietary concentration in the order of 5 ppb and beyond. These studies explicitly considered carcinogenic or mutagenic properties of chemicals. 709 substances of the carcinogenic potency database, CPDB (Gold *et al.*, 1984) were analyzed in the study.

Starting with the carcinogenic potency (= 0.5/ TD_{50}), 7 (seven) was added to the log potency to create an adjusted log value (ALV) and ensure that all low values are greater than zero. Linear extrapolation to low dose was used to estimate the dose corresponding to an upper-bound limit of lifetime risk of 10^{-6}. When these doses are plotted on a semi-logarithmic scale, they form a normal distribution with the most likely potency corresponding to a certain dose in the daily diet. In a next step, the carcinogens were divided into several subsets based on results on genotoxicity in the Ames assay, structural alert classes for carcinogenicity and LD_{50} values from short-term toxicity tests. To compare the distributions of the resulting subsets, the median adjusted log value (MALV) was calculated. Based on the result of the MALV analysis the following tiered TTC approach considering structural information and toxicological information was proposed:

- 1.5 µg/person/day (0.5 ppb): General threshold (exempting high potent carcinogens like e.g. N-nitroso or benzidine like chemicals).
- 15 µg/person/day (5 ppb): Threshold for chemicals without structural alerts for carcinogenicity or with negative mutagenicity test (Ames test).
- 45 µg/person/day (10-15 ppb): Threshold for chemicals without structural alerts for carcinogenicity or with negative mutagenicity test (Ames test) and with an appropriate acute toxicity test with LD_{50} >1000 mg/kg bodyweight. A range is offered here because the actual threshold level could depend on the LD_{50} of the particular substance.
- If a substance under review is genotoxic, the next step is to, in a case-by-case manner as is currently done under the U.S. FDA's threshold of regulation process, determine the biological relevance of the structural alert or test data.

In 1999 the EU Scientific Committee for Food presented an opinion on a program for the evaluation of flavouring substances based on the decision tree approach for risk assessment of flavouring substances in

food (**EC, 1999**). This Procedure for safety evaluation is used by EFSA (European Food and Safety Authority) when evaluation approximately 3000 flavouring substances. The human exposure thresholds adopted by the European Commission for flavouring substances are 90, 540, and 1800 μg per person and day, respectively **(EC, 2000**), based on Cramer *et al*., 1978; JECFA, 1997; JECFA, 1998; Munro *et al*., 1996; Munro *et al.*, 1999. The procedure is however not to be applied to flavourings with existing unresolved problems of toxicity, for instance substances with known or suspected genotoxic potential.

To address the questions raised by the European Scientific Committee for Food (EC, 1998b) about the applicability of the TTC to chemicals present in the diet ILSI Europe (International Life Sciences Institute) established an expert group on TTC in 1996. The goals of the group were to examine proposals for a TTC, to expand previous analysis of the concept, to develop and analyse updated databases for specific endpoints and to investigate the applicability of the TTC concept to flavours and packaging migrants, as well as to a broader range of chemicals present in the diet. The work of the expert group is presented by **Kroes *et al. (*2000)** in an examination of the possibility of defining a TTC for chemical substances present in the diet for general toxicity endpoints (including carcinogenicity), and for specific endpoints, such as neurotoxicity, developmental neurotoxicity, immunotoxicity and developmental toxicity. The study was conducted in order to determine if the TTC of 1.5 μg/person/day adequately covers non-cancer toxicological endpoints. For each of these endpoints, a database of specific no-observed-effect levels (NOELs) was compiled by screening oral toxicity studies. Endocrine toxicity and allergenicity were also addressed as two separate cases, using different approaches and methodology.

The analysis of the databases indicated the following:

- Within the limitation of the database the distribution of immune NOELs for the group of immunotoxicants examined did not differ from the distribution of non-specific endpoints NOELs for the same compound, showing that immunotoxicity should not be considered as an endpoint that is more sensitive than other non-cancer endpoints.
- For the evaluation of the endocrine toxicity endpoint the data currently available do 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. The traditional threshold approach has never been applied to food allergy, nor has a NOEL based on allergy ever been established. Allergic risks for such substances are therefore usually controlled by other means, i.e. labelling. More data are necessary to determine threshold doses for food allergens.

- Developmental neurotoxicity and developmental toxicity were not more sensitive than other non-specific endpoints.
- The neurotoxic compounds were judged to be included within the TTC of 1.5 μg/person/day, although the cumulative distribution of NOELs, were significantly lower than those for other non-cancer endpoints.
- None of the specific non-cancer endpoints evaluated in the present study was more sensitive than cancer.

The main conclusion of the group was that the analyses conducted show that a TTC 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. It was stated that the TTC 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 by the expert group. Endpoints for which validated methods had yet to be developed are e.g. endocrine activity and allergenicity.

In 1999 ILSI Europe organized an interactive meeting on the TTC concept with invited experts (**ILSI, 1999; Barlow *et al.*, 2001**). The objectives of the workshop were to communicate principles and assumptions of the TTC concept to a wider audience, to review the results of the ILSI Europe expert group's work, to provide additional scientific input supporting the validation of the concept and to consider the applicability of such a concept to other chemicals present in foods in small concentrations (such as contaminants and naturally occurring substances). In the presentation from the workshop different approaches of the TTC concept are described (the U.S. FDA Threshold of Regulation, TTC in relation to structural classes and TTC used for evaluation of flavouring substances by JECFA), and the application of TTC to some potentially sensitive endpoints (immunotoxicity, developmental toxicity, neurotoxicity and developmental neurotoxicity, endocrine active compounds, and allergenicity) were discussed.

The closing remarks of the workshop were:

- The TTC concept was considered useful in addressing limited resources on chemicals of concern. It was generally felt that the databases assembled were probably sufficient to guide the setting of TTC levels in principle.
- Since the TTC is a probabilistic concept outliers below an adopted TTC cannot be excluded. The lower the TTC the less likely the outliers; but the less it would serve to save resources.
- Further efforts are important for investigating particular endpoints such as immunotoxicity and allergenicity. For endocrine toxicity work was said to be under way and need to be evaluated before conclusions can be drawn.

- The TTC concept could become more acceptable to both scientists and risk managers if a scientifically based pre-screening assessment is developed, aimed at the earlier elimination of potential outliers. Such pre-screening might include consideration of any information from related chemicals and should include the potential for biopersistence, bioaccumulation, genotoxicity, organophosphorous-type neurotoxicity and allergenicity.
- Use of the TTC concept for regulatory purposes should be accompanied by increased sophistication of exposure assessments and considerations for both dietary and non-dietary application. In the light of new scientific data the proposed threshold values might be changed to other and even higher figures. Ranges, rather than a single figure, might become appropriate to use for TTC.

In 2005 ILSI Europe published a monograph on the TTC as a tool for assessing substances of unknown toxicity present at low levels in the diet (**Barlow, 2005**). Besides the use of the TTC concept in risk assessment of food contact materials and flavourings a wider use of the TTC concept was discussed.

Grob (2002) described the challenge with analysis of migrates from food-packing materials and lists analytical requirements and problems to be dealt with. Frequently we may ingest more than 100 μg of an unidentified migrant from a single packed food. It is concluded that many food-packing materials may not correspond to the safety called for by law, and that analysis down to the TTC (1.5 μg/person/day) seams difficult or impossible. It is therefore suggested to harness powerful analytical technology to identify at least the major migrants.

In the second report on the harmonisation of risk assessment procedures by the European Commission (**EC, 2003c**) low dose effects and thresholds of toxicological concern was discussed. Adoption of the TTC approach would be in keeping with the aim of the Commission of reducing animal use for testing purposes and avoid 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 stressors for risk assessment.

In a review article of the TTC concept, **Kroes and Kozianowski (2002)** describe the principles and discuss applications of the concept. It is concluded that the TTC principle, if applied, will be an important tool for scientists, regulators and industry. For substances such as: flavours, migrating food packaging materials and other food contacts materials, processing aids and even food additives used in low levels it will accelerate the evaluation process and lead to a appropriate priority setting.

Kroes *et al.* (2004) describes a step-wise process in detail to use a decision tree to apply the TTC principle for substances present in food at

low concentrations which lack toxicity data and for which exposure analysis can provide sound intake estimates.

Some of the considerations/conclusions made on different chemicals groups or endpoints are:

- It is suggested that a TTC would not be appropriate for chemicals with structural alerts for high potency carcinogenicity, such as aflatoxin-like substances, N-nitroso-compounds, and azoxy-compounds.
- For neurotoxic compounds a TTC of 18 μg/person/day is proposed for organophosphates (according to Munro *et al.*, 1999). For non-organophosphates neurotoxicity the class III threshold (90 μg/person/day) would apply.
- Analysis of data on teratogens gave the conclusion that a separate class of teratogens would not be needed, TTCs for structural class I, II, and III will be used.
- Regarding endocrine disrupting chemicals it was concluded; in view of uncertainties, it seems premature to consider low-dose effects for endocrine disrupting chemicals in the application of a TTC.
- Regarding allergenicity of proteins and low molecular weight chemicals there are insufficient dose-response data on which a TTC can be based.
- Specific considerations of metabolism and accumulation are not necessary in the application of a TTC, except for substances such as polyhalogenated-dibenzo *p*-dioxins and related compounds or metals (extremely long half-lives, not included in the database of Munro *et al.*, 1996).

Renwick (2004) describes the JECFA (Joint FAO/WHO Expert Committee on food additives) procedure for a safety evaluation of flavouring agents. Since 1996 JECFA has evaluated the safety of 1259 flavouring substances, based on a decision tree that incorporates a series of thresholds of toxicological concern. The safety conclusions are based on predicted consequences of metabolism and if the estimated intake is above or below a threshold of toxicological concern that is relevant to the substance. Substances are allocated to one of three structural classes, and the intake is compared with a threshold of toxicological concern derived from using data from chronic and sub-chronic toxicity studies on substances in the same structural class. In the estimation of intake JECFA uses the so-called *per capita* estimate. This implies that the amount of the compound produced annually is divided across the population that may have consumed foods containing the compound.

If a substance is predicted to be metabolised to innocuous products (i.e. products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agents) there is no safety concern if the intake is below the threshold for the structural classes, but

suitable toxicity data on the compound or structural analogues are required if the intake exceeds the threshold. If such a compound were endogenous there would be no safety concern (hormones and other substances with biochemical or physiological regulatory functions are not included). If a substance is not predicted to be metabolised to innocuous products, and the intake is above the appropriate threshold for the structural classes, data must be available on the substance or closely related substances to perform a safety evaluation. If, in the latter case, there exist a NOEL for the substance which provides an adequate margin of safety under conditions of intended use, or there exist a NOEL for structurally related substances which is high enough to accommodate any perceived differences in toxicity between the substance and the related substances the substance would not be expected to be of safety concern. If this is not the case an additional threshold of 1.5 μg/person/day, derived from doses of investigated chemicals giving a calculated cancer risk of one in a million, is applied. If the intake is below this threshold the substance will not be expected to be of safety concern, while if it is above the threshold additional data is required.

Smith *et al.* (2004) discusses a 12-step procedure for the safety evaluation of Natural Flavour Complexes (NFCs). NFCs are chemical mixtures obtained by applying physical separation methods to botanical sources. Many NFCs are derived from foods. This procedure begins with a description of the chemical composition of the commercial product, followed by a review of the data on the history of dietary use. Each known constituent of a NFC is classified in structural class I, II or II according to Cramer *et al*.,(1978); each structural class with its own TTC, 1800, 540 and 90 μg/person/day. They are also assigned to one of 33 congeneric groups of structurally related substances, on the basis of established data expected to exhibit similar pathways for metabolism and excretion as well as common toxicological endpoints (EC, 2000). The group of substances of unknown structure is placed in the class of greatest toxic potential. In subsequent steps, for each congeneric group the procedure determines the *per capita* intake, considers metabolic pathways and explores the need and availability of toxicological data. Additional toxicological and analytical data may be required for a comprehensive safety evaluation. The procedure concludes with an evaluation of the NFC in its entirety, also considering combined exposure to congeneric groups. It is concluded that the first experiences of the Flavor and Extract Manufacturers Association (FEMA) Expert Panel with the use of this procedure are very promising, but the experiences are still too few to warrant final conclusions about the usefulness of this new procedure for the safety evaluation of NFCs. Future safety evaluations of larger numbers of NFCs will indicate the usefulness of the system, either in its present form or in a form modified on the basis of experience.

Smith *et al.* (2005) has developed a guide to a step-wise procedure, which provides a chemically based approach to the safety evaluation of naturally occurring mixtures, particularly essential oils, for their intended use as flavor ingredients. The approach depends on attempted complete quantitative analysis of chemical constituents in the essential oil intended for commerce. The chemical constituents are assigned to well-defined congeneric groups that are established based on biochemical and toxicological information. Metabolic and toxicological data for each congeneric group is evaluated in the context of intake of the congeneric group resulting from consumption of the essential oil. The intake of unidentified constituents is evaluated in the context of the consumption of the essential oil as a food, a highly conservative toxicologic threshold (structural class I, II or II according to Cramer *et al. (*1978)), and toxicity data on the essential oil or an essential oil of similar chemical composition. High intake of major congeneric groups of low toxicological concern will be evaluated along with low intake of minor congeneric groups of significant toxicological concern (i.e., higher structural class). The overall objective of the guide is to organize and prioritise the chemical constituents of an essential oil in order that no reasonably possible significant risk associated with the intake of essential oil goes unevaluated.

ECETOC (2004) is proposing a concept of generic threshold values based on hazard categories (Figure 1) primarily intended to be used in the risk assessment procedure of industrial chemicals within REACH (EC, 2003a;b). 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 (EC, 1967). To derive the GEVs (Generic Exposure Values) for occupational exposure, OELs (Occupational Exposure Limits) from acute and repeated dose toxicity for in total 63 organic and non-organic substances, both volatile and non-volatile were used. For consumers exposure the GLEVs (Generic Lowest Effect Value) are based on the classification limit (50 mg/kg/d; R48 "danger of serious damage to health by prolonged exposure") for repeated dose toxicity. None of the values includes category 1 and 2 carcinogens, mutagens and reproductive toxins.

The GLEVs are suggested to be used in tiered processes of consumer risk assessment as an estimate of the actual LOAEL for the substance's repeated dose toxicity. An assessment factor of 240 is applied to take into consideration: extrapolation from LOAEL to NOAEL, extrapolation from sub-chronic to chronic study, inter- and intraspecies variation.

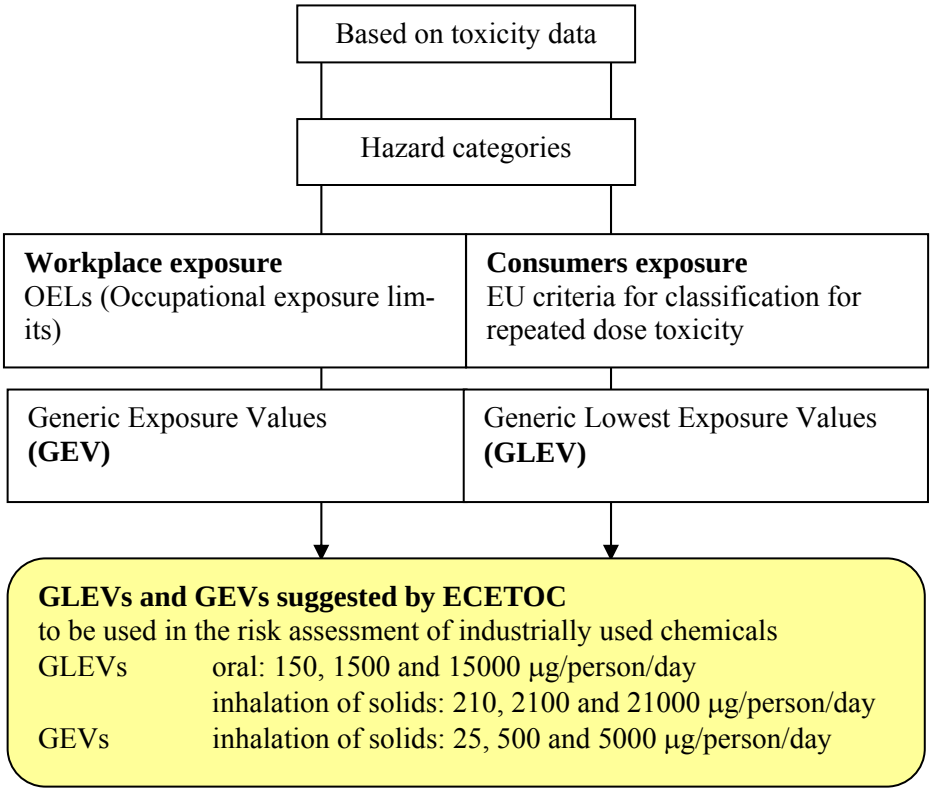

Figure 1. Use of the TTC concept as suggested by ECETOC (2004).
N.B. To facilitate comparison with values of TTC in Figure 2 the GLEVs and GEVs above have been calculated based on values given in the ECETOC report.

Consequently, for oral intake the GLEVs are: 0.5, 5, 50 mg/kg/d, 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, 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.

In the derivation of GEVs it should be noted that the OELs include socio-economic and technical arguments in addition to the scientific toxicological information, 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.

In a document produced within RIP 3.3.1-Information requirements (**Veenstra and Kroese, 2005**) the concept of TTC is discussed. It is mentioned that the TTC concept has been incorporated in the risk assessment processes in a number of regulatory schemes as a tool to justify waiving or generation of animal data. In contrast to read across or chemical categorisation, the use of the TTC is not focused or limited to the identification of potential hazards but also provides a quantitative estimate of potency. It is stated that TTC is not developed for endpoints associated with direct contact such as irritation or sensitisation, and that the following structural characteristics/properties needs special attention: non-essential, heavy metals and polyhalogenated dibenzodioxins, -dibenzofurans, or -biphenyls and similar substances, genotoxic carcinogens, organophosphates, proteins. The conclusion from this is that 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.

EMEA/CPMP (2004), Committee for Medicinal Products for Human use (CHMP), have in a "Guideline on the limits of genotoxic substances" proposed a general framework and practical approaches on how to deal with genotoxic impurities in drug substances and excipients. The application of a TTC value in the assessment is proposed. This proposal has been on external review and has not yet been decided on.

For the determination of acceptable levels of exposure to genotoxic carcinogens considerations of possible mechanism of action and of the dose-response relationship are important components. It is suggested that genotoxic impurities may be divided in two classes, "Genotoxic compounds with sufficient (experimental) evidence for a threshold-related mechanism" and "Genotoxic compounds without sufficient (experimental) evidence for a threshold-related mechanism". For compounds with clear evidence for a threshold genotoxicity, exposure levels that are without appreciable risk of genotoxicity can be established. For compounds without clear evidence for a threshold genotoxicity the assessment of acceptability should include both pharmaceutical and toxicological evaluations. There should be a sufficient justification for the unavoidability of the presence of the genotoxic impurities. The impossibility of defining a safe exposure level requires implementation of a concept of an acceptable risk level, i.e. an estimate of daily human exposure at and below which there is a negligible risk to human health. In most case only limited data from in vitro studies are available. A pragmatic approach, which recognises that the presence of very low levels of genotoxic impurities is not associated with an unacceptable risk, is therefore needed. Further analysis of subsets of high potency carcinogens led to the suggestion of a 10-fold lower TTC (0.15 μg/person/day) for chemicals with structural alerts that raise concern for potential genotoxicity (Kroes et al.,

2004). However, for application of a TTC in the assessment of acceptable limits of genotoxic impurities in drug substances a value of 1.5 μg/person/day, corresponding to 10-5 lifetime risk of cancer can be justified as for pharmaceuticals a benefit exists. Some high potency structural groups (Cheeseman et al., 1999 ; Kroes et al., 2004) require compound-specific toxicity data. The TTC value may vary according to the profile of the genotoxicity results. A TTC-value higher than 1.5 μg/person/day may be acceptable under certain conditions (e.g. short-term exposure, treatment of a life-threatening condition where safer alternatives are not available, life expectancy less than 5 years, if the impurity is a known substance and the exposure will be greater from other sources).

2.2 Regulatory use

Today, there is no regulatory experience available of the applicability of TTC on industrial chemicals. The concept is only used in the risk assessment of substances for specific use such as food contact articles and flavouring substances. The regulatory use of the TTC concept is illustrated and summarized in Figure 2.

The general threshold value used by the U.S. FDA for regulatory purpose for food contact articles is 1.5 μg/person/day (U.S. FDA, 1995). Concerning flavouring substances the TTC values used for regulatory purposes are 90, 540 and 1800 μg/person/day (EC, 2000; JECFA, 1995). In addition to these values JECFA uses the value of 1.5 μg per person and day.

In comparison, in the EU drinking water directive, limits lower than the U.S. FDA's Threshold of regulation value for indirect food additives (0.5 μg/kg in the diet) are already prescribed (EC, 1998a).

2.3 Applicability in REACH

The driving force within REACH (EC, 2003a;b) is to obtain data on a specific substance in order to be able to classify, and to assess and control the risks on substances that are classified. Classification and hazard assessment of chemicals is based on knowledge of intrinsic properties, mainly derived from testing in animals according to the information requirements in Annex V-VIII within the REACH proposal (EC, 2003b). If a substance is not classified based on data requested according at least to Annex VI and other available data, no exposure information is

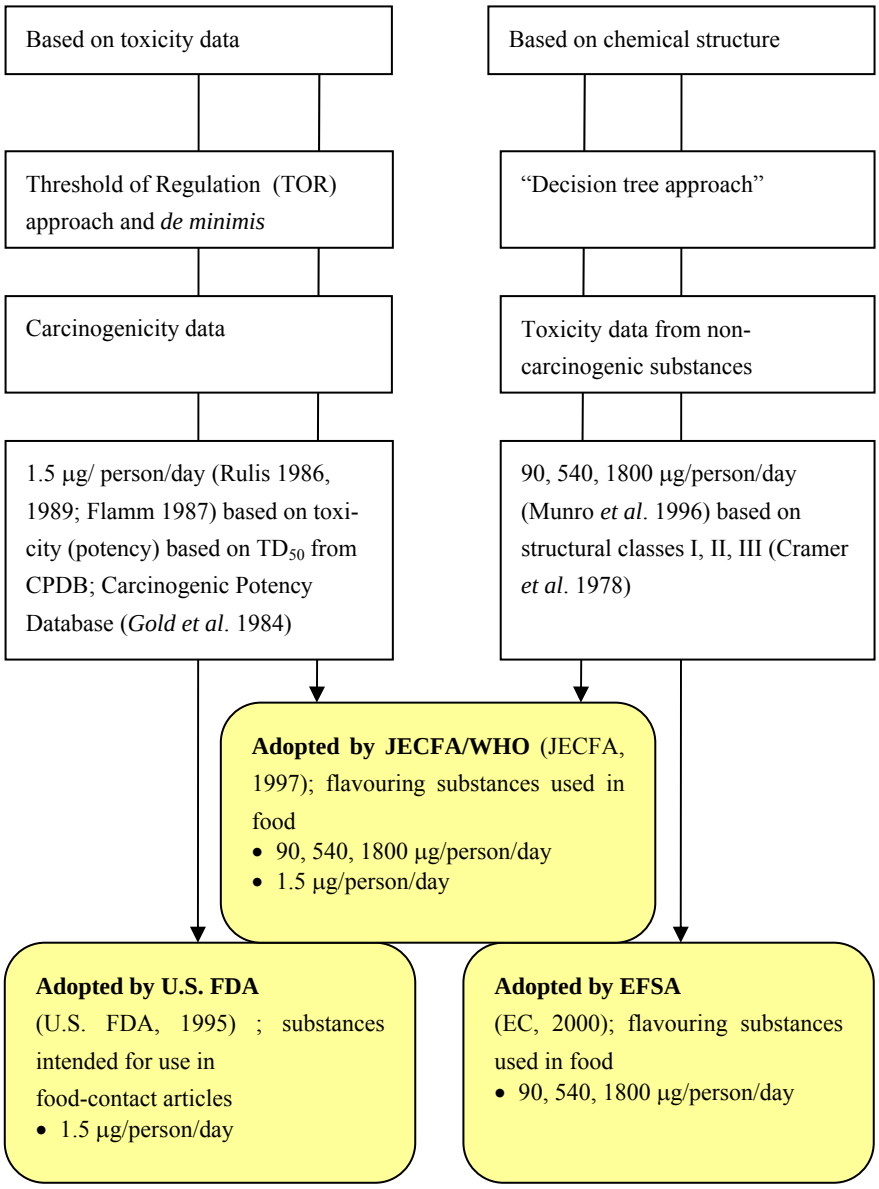

Figure 2. Principal approaches and current regulatory use of the Threshold of Toxicological Concern

requested and no risk assessment has to be performed. Thus, the TTC concept is not applicable at levels below 100 tpa because of the classification requirements and because waiving according to Annex IX is possible only at tonnage levels at or above 100 tpa. Furthermore, the use of the TTC concept prerequisites that exposure data is available.

If it is considered appropriate to use the TTC concept in REACH at levels at or above 100 tpa it might either be used as a replacement of an effect value or as the exposure limit in connection with waiving based on exposure information.

- *Effect value* A generic threshold value may in theory be used instead of a substance specific threshold value (e.g. NOAEL). Thus, it might be possible to use the TTC concept within REACH for classified substances if the actual exposure(s) is lower than the TTC value. In principle, this is also true for unclassified substances if the exposure information is available, although not requested. This implies that use of the TTC concept in this context may be seen as a driving force for deriving exposure information.
- *Exposure limit* The tests requested according to Annexes VII to VIII in the REACH proposal (EC, 2003b) can be omitted based on exposure information according to Annex IX and/or column 2 in Annexes VII and VIII. The tests requested in Annex VII are 90-days sub-chronic study and reproductive toxicity studies (in accordance with OECD TG 414 and 416). According to Annex VII, column 2, the sub-chronic study can be waived if: 1) the substance is classified based on a 28-days study or; 2) there is already a sub chronic study available or; 3) "if the substance is unreactive, insoluble and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28-days "limit test", particularly if such a pattern is coupled with limited exposure". "Limited exposure" might be a numerical value (such as an estimated value or a generic threshold value) or an exposure pattern. In Annex VIII, column 2, the corresponding "no, or no significant human exposure" is arguments to be used for waiving of the 2-generation reproductive toxicity study. Significant exposure might be regarded as a level of exposure.

2.4 Discussion

Reasons for using the TTC concept could be to achieve a more effective use of toxicological testing (reduction in the use of animals) and other evaluation resources. Thus, if appropriately derived and used the TTC concept might imply a better focus on chemicals at risk. However, there are a number of limitations or drawbacks that should be taken into consideration in deciding if the concept is to be used.

The consequences of the assumptions of toxicological, statistical and/or uncertainty factors made in the derivations of the TTCs are difficult to overview since there are uncertainties and drawbacks in more or less all of the available TTC approaches. The approaches are all based on assumptions, such as: inclusion and exclusion of e.g. specific substances, chemical structures and endpoints. In addition, there are a number of statistical assumptions for example on distribution, and choice of a risk limit. Further, the TTC values are based on the assumptions that the underlying databases (the Gold database, EU classification limits, OELs, etc.) are valid for the derivation of TTC. In the derivation of TTCs diffe-

rent assessment factors have been applied. From the investigations performed it has been concluded that all types of substances cannot be included in a concept using TTC. Substances that have been suggested for exclusion are for instance: proteins, heavy metals, polyhalogenated-dibenzodioxins, aflatoxin-like substances, N-nitroso-compounds, alpha-nitro furyl compounds and hydrazins-, triazenes-, azides-, and azoxy-compounds. In addition, the concept may be inappropriate, by itself, for chemicals that are persistent and bioaccumulate. However, there is no evidence that all potential groups to be excluded have been identified.

A number of studies has been undertaken to investigate whether different endpoints of concern, which may give rise to effects at low doses, like immunotoxic, endocrinologic, neurotoxic and developmentally toxic events could be included in the TTC values. Regarding endocrine disrupting chemicals, in view of uncertainties, it seems premature to consider low-dose effects for endocrine disrupting chemicals in the application of a TTC, and regarding allergenicity of proteins and low molecular weight chemicals there are insufficient dose-response data on which a TTC can be based. In addition, for neurotoxic compounds a general TTC does not include organophosphates. Instead a separate lower TTC was proposed. For non-organophosphates neurotoxicity the class III threshold (90 μg/person/day) would apply. Further, analysis of data on teratogens gave the conclusion that a separate class of teratogens would not be needed, thus TTCs for structural class I, II, and III could be used.

It should be noted that all endpoints have not been adequately covered and in addition that insufficient number of chemicals may have been studied for a complete evaluation.

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 TTC 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 reasonable well based with respect to general toxicity and the particular endpoints examined. However, the possible application of TTC on industrial chemicals needs to be thoroughly looked into, since there are some important differences between industrial chemicals and food contact articles/flavouring substances such as differences in use pattern, and type of chemical. Whilst the use pattern of industrial chemicals can often be characterised as wide and dispersive, the use of flavouring substances and substances in food contact articles are very specific. Furthermore, in contrast to e.g. flavouring substances, industrial chemicals are diverse and often of complex nature.

In order to be sure of protective TTC-levels the values would be rather small. Using rather crude or conservative exposure estimates (e.g. worst case scenarios and modelling) would usually be at a quantitative higher

level. This combination would probably lead to limit the use of the TTC approach to a great extent in the context with REACH.

The ECETOC approach for industrial chemicals comprises the use of generic threshold values based on hazard categories. Inclusion in hazard categories depends on the substance's specific classification (or no classification). For consumers the generic lowest effect values (GLEVs) are based on EU criteria for the classification of the substance for repeated dose toxicity (Risk phrase R48) and adjusted with an assessment factor. Further, for occupational exposure Generic Exposure Values (GEVs) are derived from OELs after adjustment with an assessment factor. It should be noted that the scientific toxicological information these OELs includes socio-economic and technical arguments.

One might question the ECETOC approach where the reasoning for using the classification limit for R48 as the numerical starting point for calculating TTC-levels is rather unclear and maybe not especially relevant as a starting point. Other things that should be taken into consideration is that up to now there is no experience of hazard categorization, and that the classification limits are effect values and not "no effects values". Furthermore, 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 material and flavourings) is expanded to be used for all kinds of substances (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.

TTC is up to now only developed and used for systemic effects from oral exposure (dietary uptake). Several toxic endpoints are therefore not covered by the TTC concept. Further consideration is needed on the use for other routes of exposure as well as how to deal with combined exposure from different routes of exposure. For industrial chemicals the predominant exposure is to workers and consumers via inhalation or by skin contact. Toxic endpoints, such as irritation and sensitisation relevant for skin and lung, were not covered by the TTC concept. Furthermore, effects caused for example by endocrine disruptive compounds are not covered.

Exposure of industrial chemicals includes occupational and consumer's exposure, as well as man exposed via the environment. It should be high-lighted that TTC values are intended to be used for the general population but up-to-date no considerations to vulnerable subgroups such as children, the elderly and pregnant women, etc. has been made. The problem with exposure to the same substance from multiple sources (occupational and consumers exposure, as well as man exposed via the environment) is a general problem and not solved by the use of TTC.

The use of the TTC approach is dependent on rather precise quantitative exposure estimates. Experiences from the EU Risk Assessment Pro-

gramme for Existing Substances are that it is very difficult to get sufficient information on the different uses and related exposure to make precise exposure estimates and before any experience from REACH is available it is difficult to foresee any improvement.

Precise exposure estimates can only be done in case very detailed exposure data are available. For substances where only very limited (or no) 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.

In conclusion, the use of general effects values are not applicable within REACH at levels below 100 tpa. In theory, in the decision whether toxicity studies may be omitted at levels at or above 100 tpa is appropriate or not a general effect value (such as TTC) might be used in the comparison with the available exposure information. However, due to limitations and uncertainties in the derivation of TTCs as well as to the fact that the TTC concept has not yet been evaluated for the diverse group of industrial chemicals and for the different routes of exposure other than dietary we consider it too premature to use the concept within REACH.

Finally, it is of utmost importance to obtain a sufficient level of protection in risk assessment of industrial chemicals and it is doubtful whether this is possible to achieve by the use of the TTC concept.

3. Environment

3.1 Literature review

Two different approaches have been used when deriving a TTC for the environment, i.e. the "action-limit" proposed by EMEA/CPMP (2001) and the environmental Exposure Threshold of No Concern (ETNC) proposed by ECETOC (2004) and de Wolf *et al*. (2005). Both these approaches are restricted to the pelagic freshwater compartment. The approaches are described briefly here, while more specific information is presented in the literature review below.

The first of these TTC-approaches, i.e. the "action-limit", originates from a draft on environmental risk assessment of human pharmaceuticals (EMEA/CPMP, 2001), describing a tiered risk assessment process. The initial step is an environmental exposure assessment in which a coarsely predicted environmental freshwater concentration (PEC) for the pharmaceutical ingredient, or its major metabolites, is compared to an action-limit (0.01 μg/L). In case the PEC is smaller than the action-limit and no environmental concerns are apparent, no further action is considered needed. On the other hand, when the PEC is larger than the action-limit, the assessment continues to a second phase, which involves an environmental fate and effect analysis.

The action limit is based on an aquatic concentration below which it was concluded that no ecotoxicity data on drugs for relevant standard test organisms were reported (U.S. FDA, 1996). This concentration was further divided by an assessment factor of 100 to obtain the action limit.

The CSTEE (2001) did not find this "action-limit", 0.01 μg/L (proposed by the EMEA/CPMP), being scientifically valid, since pharmaceuticals with lower effect concentrations were found. They also criticized the approach presented in the draft being focused on acute toxicity, which is not considered to provide the most appropriate basis for risk assessment given the intended specific mode of toxic action/potency of pharmaceuticals in general.

A different TTC-approach was applied deriving an ETNC for the pelagic freshwater compartment, i.e. $ETNC_{aquatic}$ (ECETOC, 2004; de Wolf *et al*., 2005). This approach was based on existing toxicological databases and substance hazard assessments for organisms in the freshwater environment, and a categorisation of chemicals into four different modes of action (MOA). Two main databases with 53 and 254 substances, respectively, and two supportive databases with 412 and 138 substances, respectively, were used. The stratified data was fitted to a lognormal distribution from which a fifth percentile, with a 50% confidence interval, was

determined. This value was then divided by an assessment factor, ranging from 1 to 1000 depending on the data (see details below), to obtain the $ETNC_{aquatic}$.

The four different modes of action used were according to the system by Verhaar *et al*. (1992): MOA1 = inert chemicals (baseline toxicity, narcosis); MOA2 = less inert chemicals (acting by polar narcosis); MOA3 = reactive chemicals (react unselectively with certain chemical structures in biomolecules); MOA4 = specifically acting chemicals (specific or receptor toxicity). Metals, inorganics, and ionisable organic chemicals are not covered by this system, and thus not included when deriving the $ETNC_{aquatic}$.

The resulting $ETNC_{aquatic}$ for MOA1 ranged between 1.5 and 5 µg/L, depending on data source. The corresponding ranges for MOA2, MOA3 and MOA1-3 were: 1.4-12, 0.07-0.11 and 0.09-0.28 µg/L respectively. The authors proposed an overall value of 0.1µg/L for MOA1-3. The authors considered that a broad application of the $ETNC_{aquatic}$ concept also needed to cover MOA4, and that the resulting $ETNC_{aquatic}$ likely would have to be much lower. This idea is substantiated by the fact that a substantially lower $ETNC_{aquatic}$ was observed when analysing the chemicals assigned a MOA4, as the resulting $ETNC_{aquatic,\ MOA4}$ was 0.0004 µg/L. The lowest individual NOEC value in that particular database was 0.0006 µg/L (Fenthion).

The authors (de Wolf *et al.*, 2005) suggest that the $ETNC_{aquatic}$ only should be used as a first tier of assessment, in the absence of any effect data, and may as such be helpful for chemical producers and importers in order to set data-generating priorities, downstream users about the relative risk associated with their specific uses, and that it also may be valuable in putting environmental monitoring data into a risk-assessment perspective.

3.1.1 Cited references

CSTEE (2001) This document includes the CSTEE opinion on the Committee for Proprietary Medical Products (CPMP) of the European Agency for Evaluation of Medical Products (EMEA) draft document. As regards the TTC concept; the CSTEE did not find the trigger concentration approach (0.01 µg/L), proposed by the CPMP, being scientifically valid, as pharmaceuticals with lower effect concentrations were found. They also criticized the approach presented in the draft being focused on acute toxicity, which is not considered to provide the most appropriate basis for risk assessment given the intended specific mode of toxic action/potency of pharmaceuticals in general.

de Wolf *et al.* (2005) In this document an Environmental Threshold of No toxicological Concern for freshwater systems ($ETNC_{aquatic}$) is derived.

It is approximately 0.1 μg/L for organic chemicals not exhibiting a specific mode of action.

The authors analysed four environmental toxicological databases (Existing substances risk assessment, ECETOC aquatic hazard-assessment database, the U.S. EPA Fathead minnow database, and the Guppy database from the Utrecht University) and derived the lowest effect value and the lowest 5th-percentile values from each of these databases using data stratification based on mode of action (MOA; 1 = inert chemicals; 2 less inert chemicals; 3 = reactive chemicals; 4 = specifically acting chemicals). The $ETNC_{aquatic}$ values were derived dividing the 5th percentile with application factors. According to the TGD an assessment factor of 10 is applied only when long-term toxicity NOEC values are available from at least three species across three trophic levels (e.g. fish, Daphnia, and algae). In deriving $ETNC_{aquatic}$ from the ECETOC database an assessment factor of 10 was used as it was assumed by the authors that this TGD condition was fulfilled. When deriving $ETNC_{aquatic}$ for acute toxicity (i.e. the Fathead minnow and the Guppy databases) an assessment factor of 100 was used for MOA1 and MOA2, and an assessment factor of 1000 for MOA3 substances and in case of multiple MOAs. A narrow species-sensitivity distribution and small Acute Chronic Ratios (ACRs; references given in article) were considered to justify an assessment factor of 100 for substances exhibiting narcosis-type toxicity.

It is unclear from the article if i) only one value (the lowest?) for each substance was used when deriving the $ETNC_{aquatic}$ for the different databases, and ii) if the assumption regarding the NOEC-values for all substances in the ECETOC database was correct. The conclusions drawn from this study depends on the answers to these questions.

In addition, the authors refer to a study by Straub (2002), who was supposed to have applied the $ETNC_{aquatic}$ concept to pharmaceuticals (classed as MOA4 substances) and proposed a value of 0.01 μg/L as being appropriate for aquatic organisms. The authors use this value, i.e. 0.01 μg/L, when assuming that the $ETNC_{aquatic}$ for the other MOAs (MOA1, MOA2, and MOA3) should be higher, as these types of chemicals not specifically will interact with biota. However, Straub did not propose a value of 0.01 μg/L as appropriate for aquatic organisms, nor did he apply the $ETNC_{aquatic}$ concept to pharmaceuticals. Straub commented upon the EMEA/CPMP draft (2001), which was the document where the concentration of 0.01 μg/L actually was mentioned and proposed as an action limit.

EMEA/CPMP (2001) This document includes a proposal for a stepwise phased procedure for assessing the potential risks to the environment of medical products for human use. If the Phase I PECsurface water value (predicted concentration of the substance in surface water) is below 0.01 μg/L, and no other environmental concerns are apparent, the medi-

cinal product is not further considered. If not, a described Phase II environmental effect analysis should be performed.

The basis for the proposed action limit of 0.01 μg/L is not clear. It is however stated in the most recent version of this draft that "The present action limit is based mainly on acute toxicity data and will therefore be revised when a sufficient amount of chronic data exists" (EMEA/CPMP, 2005). According to the Swedish Medical Protection Agency (Personal communication with A-K Johansson, Swedish Medical Protection Agency, 2005) the origin of the proposed action limit is a concentration from the U.S. FDA (1996) document (described below), below which no toxicity was observed, divided by an assessment factor of 100.

EMEA/CPMP (2005) Most recent version of this draft. Action limit of 0.01 μg/L still included.

ECETOC (2004) This document basically contains the text published as the article by de Wolf *et al*. (2005). The comments given on that article thus holds also for this reference.

U.S. FDA (1996) This document provides the ecotoxicity value for pharmaceuticals, which divided with an assessment factor of 100 results in the action limit of 0.01 μg/L used in the EMEA/CPMP (2001).

The database examined consisted of 278 ecotoxicity test results, mostly acute toxicity data, for 76 different drugs. The data were examined to determine a level at which significant effects on relevant standard test organisms were not routinely observed. This was done by analysis of no observed effect concentration (NOEC), minimum inhibitory concentration (MIC), and/or lowest observed effect concentration (LOEC) determined by testing. If only an EC_{50}/LC_{50} were available, the NOEC was predicted by dividing the EC_{50}/LC_{50} with an assessment factor of 1000. The data were not statistically analyzed as "…a conservative approach was used to establish the no concern level, i.e., a level was chosen at which effects were not observed or expected to be observed."

No experimentally derived test data for relevant standard test organisms were reported below 1 μg/L. Including also the calculated NOECs resulted in one result, out of a total of 278 test results, which was lower than 1 μg/L. An anesthetic (Midazolam) had no definitive NOEC determined by testing, but instead an EC_{50} of 200 μg/L, which divided with an assessment factor of 1000, resulted in a calculated NOEC of 0.2 μg/L. It was concluded that drugs at concentrations less than 1 μg/L in the aquatic environment ordinarily have no significant effect on relevant standard test organisms.

Straub (2002) This document comments upon the environmental risk assessment proposed in the EMEA/CPMP draft (2001). The author does not, as have been wrongly cited in de Wolf *et al*. (2005) and ECETOC (2004), propose an Environmental Threshold of No toxicological Concern for freshwater systems ($ETNC_{aquatic}$) of 0.01 μg/L as appropriate for aquatic organisms for pharmaceuticals.

Verhaar *et al*. (1992) This document contains a scheme that makes it possible to classify a large number of organic chemicals into one of four classes, viz: (1) inert chemicals (baseline toxicity), (2) less inert chemicals, (3) reactive chemicals, (4) specifically acting chemicals. This method of classifying chemicals is used by ECETOC (2004) and de Wolf *et al*. (2005). More recent articles have revised the distinction between inert and less inert chemicals and combined them into one class, resulting in the following three classes: baseline toxicity (combining inert and less inert chemicals), reactive toxicity, and specific toxicity (Vaes *et al*., 1998; Escher and Hermens, 2002).

3.2 Regulatory use

There is presently no use of the TTC concept as regards environmental assessments. However, in a draft by EMEA/CPMP (2001, 2005) a stepwise, tiered procedure for the environmental risk assessment of pharmaceuticals (for human use) is proposed. This approach would involve a TTC approach as it includes an action limit of 0.01 µg/L in pelagic freshwater environment.

3.3 Predicted exposure scenarios

In order to evaluate the possible outcome of a use of the TTC-concept, an exposure simulation was performed in this project.

The simulation was performed using a high emitting (2%) industry category (formulating industry) varying use volume, log K_{ow}, water solubility, degradation, and dilution of wastewater. The outcome is presented in Table 2.

Dilution x10 is considered a worst-case scenario, while dilution x100 and x1000 are considered more realistic. The emission factor to wastewater for this standard emission scenario changes in EUSES from 0.02, below 1000 tpa, to 0.003 above.

It is from this exposure simulation apparent that an application of the TTC-concept, with the $ETNC_{aquatic,MOA1\text{-}3}$ = 0.1 µg/l, could open for waving of toxicity testing in REACH (EC, 2003a; 2003b). This, especially considering that the industry category used in this exercise (formulation industry) is most certainly among the higher emitting industry categories. This indicates that TTC potentially could be used for waving by several industry categories.

It is also obvious from this simulation that using a toxicity threshold calculated for MOA4, e.g. $ETNC_{aquatic,\ MOA4}$ = 0.0004 µg/l, would to a substantially lesser degree open to exposure based waving, as compared to using a toxicity threshold estimated based on MOA1-3.

Table 2: EUSES 2.0 simulations performed in this report, with different standard release conditions (Fmain = 1, Temission = 200 d, Dilution = 10-1000, STP = Yes, PEC during emission period, non-volatile substance) were performed for a standard formulation industry (Formulation A-table A2.1 (emission factor 0.02), IC = 2 (Chemical ind.)).

Volume (tpa)	Log Kow	Water solubility (mg/l)	Ready biodeg[a]	PECfreshwater (µg/l)		
				Dilution x10	Dilution x100	Dilution x1000
10	0	1000	No	50	5	0.5
10	0	1000	Yes	6.3	0.6	0.06
10	6	0.01	No	6.5	0.6	0.06
10	6	0.01	Yes	2.9	0.3	0.03
100	0	1000	No	500	50	5
100	0	1000	Yes	63	6.3	0.6
100	6	0.01	No	65	6.5	0.7
100	6	0.01	Yes	29	3	0.3
1000	0	1000	No	750	75	7.5
1000	0	1000	Yes	95	9.5	1
1000	6	0.01	No	98	9.8	1
1000	6	0.01	Yes	44	4.4	0.4

[a] "Ready biodeg = No" means no degradation, i.e. persistant

3.4 Applicability in REACH[1]

The $ETNC_{aquatic}$ value is a general $PNEC_{aquatic}$ value derived from effect data. If a substance is present in the pelagic freshwater environment in a concentration below this value, it is assumed that adverse effects in the aquatic environment are not likely to occur and that the substance should not be further assessed (cf. Risk characterisation ratio < 1). Thus, the exposure information decides whether new aquatic toxicity tests should be performed.

According to REACH, the use of exposure information as a basis for waving of tests might be considered allowed for substances produced in quantities above 100 tpa (i.e. Annex VII and VIII) (EC, 2003b). This would imply that a possible application of the $ETNC_{aquatic}$ concept would concern waiving of aquatic tests required by these two annexes, i.e. long-term tests for Daphnia and fish.[2]

1 Application of ETNC in REACH as a screening tool for prioritisation of substances for further assessment seems not to be appropriate. The substances are there already prioritised based on thier (i) production volume and (ii) intrinsic properties (Substances of Very High Concern)

2 These tests are required by Annex VII. Annex VIII does not include toxicity tests on pelagic aquatic species.

Since application of this concept could result in waiving of long-term aquatic tests, the basis for derivation of such an $ETNC_{aquatic}$ should be based on data from chronic toxicity tests.

Following standard tiered risk assessment procedures, i.e. starting with worst case assumptions (if no concern OK, otherwise refinement) it is reasonable to base derivation of a TTC on conservative assumptions. Thus, a hypothetical use of the $ETNC_{aquatic}$ should be based on results from the most toxic substances (i.e. worst case assumption), suggestively substances acting by, what Verhaar *et al*. (1992) described as specific mode of action.

Possible consequences of applying $ETNC_{aquatic}$, for waving of long term toxicity tests (Annex VII), on hazard assessment in REACH:

i. *Classification*
   Because classification criteria are based on results from short-term toxicity studies, the application of $ETNC_{aquatic}$ would not effect the classification.
ii. *Derivation of $PNEC_{aquatic}$*
   Two scenarios are possible: either (i) using the $ETNC_{aquatic}$ (which is a general $PNEC_{aquatic}$), and thus no substance specific risk assessment will be performed, or (ii) deriving a substance specific $PNEC_{aquatic}$ based on acute toxicity data (collected from the lower tonnage levels divided by an assessment factor of 1000).
iii. *Derivation of $PNEC_{sediment}$ and $PNEC_{soil}$ using the EP method*
   Two scenarios are possible: either (i) using the $ETNC_{aquatic}$ (which is a general $PNEC_{aquatic}$) as base for deriving $PNEC_{sediment}$ as $PNEC_{soil}$, and thus no substance specific risk assessment will be performed, or (ii) deriving a substance specific $PNEC_{aquatic}$ based on acute toxicity data (collected from the lower tonnage levels divided by an assessment factor of 1000) as base for deriving $PNEC_{sediment}$ as $PNEC_{soil}$.
iv. *PBT (Persistent, Bioaccumulating and Toxic) assessment*
   Lack of substance specific toxicity data could hamper the T-assessment. However, the consequences are difficult to forsee as also the P- and B-criteria have to be considered.

3.5 Discussion

The TTC-concept represents a new approach as regards environmental risk assessments since it results in a general PNEC (a non-effect threshold value) that is intended to be applied on an entire group of substances, as compared to the standard substance specific PNEC.

The TTC approach is developed only for direct effects on the pelagic freshwater ecosystem and not effects due to bioaccumulation, or accumulation in other compartments. In addition, the concept does not cover

metals, other inorganic compounds, or ionisable organic compounds. The use of the threshold of no toxicological concern, as compared to experimental data, implies a higher risk of not considering the toxicity of degradation product(s)/metabolite(s), which may be unfortunate if they are more toxic than the parent compound.

It has been proposed to use the TTC concept as a tool for screening in order to select/prioritise substances for testing/further risk assessment, e.g. it may help to inform downstream users about the relative risk associated with their specific uses. The approach could also be valuable in putting environmental monitoring data into a risk-assessment perspective. For these applications the concept may work if the TTC is satisfactory determined. However, because only toxicity is considered, P and B-criteria should also be consulted.

The main reason using the TTC approach would be the saving of aquatic freshwater test organisms, including vertebrate species (mainly fish).

Two different approaches have been found in the literature that can be seen as environmental TTC, i.e. action-limit and $ETNC_{aquatic}$.

The action-limit proposed by EMEA/CPMP (2001) has been questioned by the CSTEE since drugs with lower effect concentrations were found. In addition, the focus on acute toxicity in the draft was questioned, as chronic toxicity was considered more relevant for this kind of substances, i.e. pharmaceuticals.

The method of deriving a PNEC, using the NOEC for the most sensitive species and an assessment factor, is the standard approach in TGD to derive a threshold value for one chemical. When deriving a toxicity threshold value for substances belonging to a defined group of chemicals, the toxicity data for the most sensitive species for those chemicals are used, with the application of an assessment factor. Is the safety level for the environment similar in these two cases?

When using the $ETNC_{aquatic}$ concept, substances that are toxic at very low concentrations may slip through, i.e. those with an effect concentration below the 5^{th} percentile. The method of using a 5^{th} percentile and lognormal distribution to derive a threshold value has been accepted for individual data rich substances, e.g. Zn in the Existing Substance Regulation. In that case ecotoxicity data for a number of species was used to derive a toxicity threshold for one substance. However, for the $ETNC_{aquatic}$, data from a number of chemicals and a number of species would be used to derive a toxicity threshold value valid for all substances belonging to a defined group of chemicals. In the first case, the concept accepts that 5% of the species NOECs will fall below the threshold. In the second case, the concept accepts that 5% of the chemical PNECs will fall below the threshold. Is the safety level for the environment similar in these two cases? The consequences should be further evaluated.

What is the added value of using a generic PNEC as compared to (Q)SAR estimates, when no substance specific experimental toxicity data is available? As regards what Verhaar *et al.* (1992) defined as mode of action 1-2, available QSAR models exists, which are based on more specific data, which should be more relevant than a generic TTC. However, it should be stressed that QSARs are usually used as indicators of an effect, and not for confirmation of lack of effects (which is the opposite of how the TTC is proposed to be used!).

If the TTC-concept is to be used, should one or several threshold values be used? Using more than one threshold value implies a higher risk of using the wrong (not safe) threshold. The use of several thresholds put higher demands on the categorisation system. Chemicals may be categorised according to different systems. Considering the fact that the knowledge in this field has continued to grow over the years, is the approach suggested thirteen years ago by Verhaar *et al*. (1992), as proposed by ECETOC (2004) and de Wolf *et al*. (2005), presently the most appropriate way of grouping chemicals in order to derive a TTC? This method uses four modes of toxic action to differentiate between chemicals. Even though rules exists as to categorize that a chemical exhibits one of the first of these three modes of action, it is however not possible, based on definite structural rules, to decide whether or not a substance exhibits the fourth of these modes Inclusion in this fourth class must, and should, be based on specific knowledge on mode of toxic action of (groups of) chemicals. In addition, a substance may have more than one mode of action. Hence, the use of only one threshold value appears to be the most transparent and conservative approach. As a consequence of the above, it seems reasonable to base this threshold value on chronic toxicity data for the most toxic chemicals, i.e. those categorized as having a specific mode of toxic action.

Regarding a possible use of the TTC in REACH, the REACH proposal only opens for exposure based waving for substances > 100 tpa. The use of the TTC-concept would thus be restricted to Annexes VII and VIII.

If applied at tonnages > 100 tpa the concept may be used for waving of aquatic toxicity tests (not sediment tests) required by Annex VII (i.e. long-term toxicity for Daphnia and fish, BCF for fish). Following the REACH proposal, low water solubility trigger long-term tests at lower levels (according to REACH water solubility >1 mg/l), thus these tests should not be waived with the TTC approach.

The theoretical EUSES modelling performed above, of chemicals with different properties used in higher emitting industry categories, shows a potential for TTC based waiving (the less emitting industries would come to even lower levels, thus below the proposed TTC values). However, if the TTC value is based on chemicals acting by what Verhaar *et al*. (1992) defined as a specific mode of action, as it preferably should (see above), it is reasonable to assume that the amount of chemicals involved would

be so low that the whole idea of using this approach, as means of reducing testing, looses much of its possible attraction.

It is possible that, in order to apply the approach if real exposure data is required, the cost of requiring this exposure information very well may exceed the cost benefit from test waving.

If the TTC approach is applied within REACH at tonnages > 100 tpa the consequences will be that PNEC either will have to be derived based on short-term data only or on the TTC value, which results in a more uncertain PNEC value. In addition, it could also result in that no substance specific risk assessment will be performed. The application of the TTC concept could also influence the effect assessment in other environmental compartments. The equilibrium partitioning (EP) method could be applied, using the TTC-derived $PNEC_{aquatic}$ as the base for deriving $PNEC_{sediment}$ and $PNEC_{soil}$. The other possible consequence is that the EP estimates will be based on $PNEC_{aquatic}$ from short-term data. This is an example of how, although there are no TTC for other environmental compartments than water, the TTC concept may be (mis)used for other compartments.

Classification is based on short-term toxicity data, and thus not affected by a possible waving due to TTC, as acute toxicity test are required already in Annex V and VI, for which no exposure based waving is possible in the present REACH proposal.

TTC can presently not be used as a stand-alone concept, but could perhaps in the future be included in a weight-of-evidence approach when deciding on potential derogations.

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