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TECHNICAL REPORT OF EFSA

Outcome of the public consultation on the draft EFSA opinion on Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC)[1]

European Food Safety Authority[2]

European Food Safety Authority (EFSA), Parma, Italy

BACKGROUND

EFSA has undertaken a public consultation on a draft opinion on "Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC)". The draft opinion was prepared by an EFSA Scientific Committee working group composed of external experts, members of the Scientific Committee and the Scientific Panels. The draft opinion was endorsed for public consultation by the EFSA Scientific Committee on the 22nd of June 2011. The public consultation lasted 11 weeks, from 7th of July until the 23rd September 2011. This report provides a summary of the comments received and how they were considered by the working group of the Scientific Committee for updating the opinion. The working group held two additional meetings and one teleconference to address the comments received.

COMMENTS RECEIVED

Following the public consultation process, EFSA has received over 224 comments from 37 interested parties including academia, industry, industry organisations, non-governmental organisations, national and international agencies, assessment bodies and private individuals. The names of organisations that submitted comments are listed in Appendix A of this report. All the comments received are listed in Appendix B of this report. Comments submitted formally on behalf of an organisation appear with the name of the organisation. Comments submitted by individuals appear with no attribution. For the sake of completeness, EFSA also has taken into account submissions done by e-mail, even though this was explicitly excluded in the instruction for this Public Consultation on the EFSA website.

EFSA Panels and Units were also invited to take part of the consultation. Contributions were received from FEEDAP and ANS Panels.

The Scientific Committee working group discussed all the comments and addressed the relevant ones for updating the draft opinion. Many of the comments received were deemed appropriate and strongly contributed to enhance the scientific quality and clarity of the opinion. The opinion was revised accordingly, providing additional clarifications and explanations. Comments outside the remit of EFSA were not addressed, but are included in the table of comments.

A brief summary of the main comments received and how they were addressed by the working group is given below.

[1] On request from EFSA, Question No EFSA-Q-2008-00855, issued on 22 May 2012.

[2] Correspondence: scientific.committee@efsa.europa.eu

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SCREENING AND EVALUATION OF THE COMMENTS RECEIVED

1. GENERAL COMMENTS

Among the comments received, some expressed that there was lack of clarity in the final recommendations as to where and how EFSA intends to further apply the TTC approach in its work. Apparently it was not clear, for example, if it would be applied to regulated substances, or used only for impurities, metabolites, breakdown and degradation products, and low level contaminants.

Several comments, expressed concern that the TTC approach would be used for the active or primary ingredients of regulated products, instead of following the current requirements for submission of toxicity data. This concern was also expressed regarding pesticide metabolites.
Other comments were in favour of using the TTC approach for primary ingredients if resulting human exposures are very low. Comments also indicated there was a lack of clarity on whether the TTC approach would only be used as screening tool to prioritise substances for full evaluation, or whether it would be used as a final tool for deciding that a substance requires no further data/evaluation.

The revised opinion clarifies where EFSA recommends that the application of the TTC approach could be beneficial, as summarised in the following conclusions from the opinion:

a) The TTC approach is applicable to substances for which the chemical structure is known but for which there are few or no relevant toxicity data. For the work of EFSA, the TTC approach is recommended as a useful screening tool either for priority setting or for deciding whether exposure to a substance is so low that the probability of adverse health effects is low and that no further data are necessary.
b) In principle, the science supports the application of the TTC approach in any area of chemical risk assessment for which human exposures are low, whether exposure is from deliberate addition or due to contamination. However, for substances for which EU legislation requires the submission of toxicity data, the TTC approach would not be used.

c) Within EFSA, the Scientific Committee recommends that the TTC approach can be used to assess impurities, breakdown and reaction products, metabolites, and low-level contaminants in food and feed where an exposure assessment can be conducted, but on which there are few or no toxicological data.

d) Wider use of the TTC approach in EFSA's work, beyond the ones mentioned in (b) above, can also be envisaged, for example, as part of tiered approaches in which toxicity testing requirements are linked to the level of human exposure. Such uses should be considered on a case-by-case basis, in consultation with risk managers. The Scientific Committee further recommends that in such cases, if there is a structural alert for genotoxicity, then genotoxicity testing data on the substance or information (e.g. from read-across) should be sought.

e) The Scientific Committee recognises that when the different EFSA Panels apply the TTC approach to their respective areas, specific considerations may apply and the generic scheme may need to be adapted, as has been done in the case of flavouring substances.

2. SPECIFIC COMMENTS:

2.1. Comments related to risk management aspects

Some comments asked whether recommending use of the TTC approach requires discussions with risk managers since the use of a probability-based method implies the acceptance of some degree of risk, albeit a low probability of risk, and such decisions are a matter for risk managers.

It was agreed that decisions on the acceptability of low level risks is indeed a matter for risk managers, but that discussions were needed between risk assessors and risk managers. It was noted, for example, that risk managers need advice from risk assessors on the scientific aspects of any uncertainties in the TTC approach. The Scientific Committee discussed the treatment of uncertainty in the way TTC values have been derived and was of the view that the opinion does not stray across the boundary between risk assessment and risk management.

2.2. Comments related to the degree of conservatism in the TTC approach

Several comments questioned the extent to which TTC values for cancer and non-cancer endpoints can be considered to be adequately conservative. In particular, for non-cancer endpoints, it was questioned why the 5th percentile NOELs are used rather than the lowest NOEL in each Cramer Class to derive the relevant TTC value.

For cancer endpoints, there is wide agreement in the scientific community that the use of linear extrapolation from animal cancer bioassays to estimate human health risks at low exposures yield very conservative estimates, and since that method was used in deriving TTC values for genotoxicity/cancer endpoints, they are conservative, irrespective of the fact that not all scientists agree on the accuracy of estimates derived by linear extrapolation. It should also be noted that in the generic scheme proposed by the Scientific Committee, only the lower (0.15 µg/person per day) of the two existing values for cancer endpoints would be used in practice for substances with a structural alert for genotoxicity, and that substances related to known high potency carcinogens are excluded from the TTC approach.

For non-cancer endpoints, it should be noted that in deriving the TTC values, the 5th percentile NOEL values *per se* are not used, rather it is the 5th percentile NOEL values divided by a safety (or uncertainty) factor of 100 that are used, thus adding a margin of safety. A default factor of 100 is widely accepted in risk assessment to take account of both interspecies differences between laboratory animals and humans and inter-individual differences between humans. It should also be noted that the derived TTC values are below the lowest NOEL for the relevant Cramer Class. The opinion has been modified to make these elements of conservatism more clear.

Finally, it should be emphasised that the TTC approach is probability-based; it can offer reasonable certainty that exposure to a substance below its relevant TTC value will be without adverse effects on human health, but it does not offer absolute certainty. The opinion now includes a quantitative estimate of the chance that a substance with an exposure below the relevant TTC value may still pose a potential risk; the probability lies between zero and 5%. In this respect, it is not different from other, data-driven risk assessments, where the extrapolation of *in vitro* or *in vivo* testing results always carries a degree of uncertainty. Comments on the adequacy of the TTC approach to cover endocrine active substances

Some comments underlined the lack of consensus in the scientific community on this issue and suggested to better reflect the diverging views in the international scientific community on the potential for low-dose effects from endocrine active substances. Some comments asserted, with examples, that the human exposure threshold values for non-cancer endpoints do not cover low-dose effects of endocrine active substances.

The Scientific Committee considered these comments in depth and revised the text and conclusions on substances with endocrine activity. The opinion now makes clear that EFSA is fully aware of the ongoing activities in the EU to develop a systematic approach for identification and assessment of endocrine disruptors, but since those activities are not yet completed, the Scientific Committee considered it was important, in the meantime, to give some advice on the use of the TTC approach in relation to this issue.

The Scientific Committee noted that the Munro et al. (1996) database underpinning the TTC approach contains apical studies that have assessed some toxicity endpoints (e.g. reproductive and endocrine organ pathology, reproductive function and embryo-fetal development) that can be affected adversely by substances with an endocrine mode of action. In addition, analysis of TTC values in relation to currently established NOELs for reproductive and developmental toxicity, based on standard testing protocols used in the past, and the data so far indicate that the TTC values are adequately protective for the types of adverse effect that such studies can detect.

The Scientific Committee noted that a number of the examples cited in one set of comments of substances with endocrine activity at low doses, included data from non-mammalian species that are not relevant to human risk assessment, and to substances that bioaccumulate, which have already been recommended to be excluded from the TTC approach. Substances with steroid structures were also raised in comments and the Scientific Committee did agree a new recommendation that they should be excluded from the TTC approach as it is known that some steroid hormones have potent endocrine (and carcinogenic) activity. The Scientific Committee also considered whether structures related to thyroid hormones or to substances known to interfere with thyroid hormones should also be excluded, but the scientific literature indicates such substances only exert effects at doses above the non-cancer TTC values.
Taking all the above into consideration, the Scientific Committee concluded as follows:

a. In most situations where the TTC approach might be applied, there would be no *a priori* knowledge that a substance has endocrine activity.
b. If there are data showing that a substance has endocrine activity, but the human relevance is unclear, then these data should be taken into consideration, case-by-case, in deciding whether or not to apply the TTC approach.
c. If there are data showing that a substance has endocrine-mediated adverse effects, then, as would be the case for adverse data on any other endpoint, the risk assessment should be based on the data, rather than the TTC approach.
d. In view of the extensive work, currently ongoing, to develop an EU-wide approach for defining and assessing endocrine disrupters, once that approach is finalised it will be necessary to consider any impact it may have on the use of TTC approach.
e. In the meantime, the Scientific Committee recommends that untested substances, other than steroids, can be evaluated using the TTC approach recommended in this opinion.

2.3. Comments related to structural classification of substances in Cramer Classes

Some comments expressed the view that the TTC value for Cramer Class III should be adjusted upwards given that a separate, lower TTC value for structures related to organophosphates (OPs) and carbamates is proposed. If OPs are removed from the underpinning Munro et al. (1996) database for Class III, the distribution of NOELs shifts and the 5th percentile value increases.

The Scientific Committee confirmed its view that, given the majority of substances fall into Cramer Class III, in order to maintain a conservative approach the TTC value for Cramer Class III should not be adjusted upwards. Such an adjustment might be appropriate in the future if the existing Munro et al. (1996) database were to be extended by addition of more recent studies and a reanalysis supported a change in the TTC value.

Some comments expressed concern at the proposal in the draft opinion not to use the TTC value for Cramer Class II since it lacks support from the few substances in the underpinning databases that fall into Class II. Clarification was requested on the implications for a large number of flavouring substances already evaluated by EFSA that fall into Cramer Class II. Some comments argued that rather than discarding Cramer Class II, the Cramer decision tree should be further refined.

Further text has now been added to the opinion to address this issue, noting that, with the exception of flavouring substances, neither the Munro et al. (1996) database nor subsequent publications by other groups using different databases have identified more than a few substances that fall into Cramer Class II. The Scientific Committee remains of the view that the TTC value for Cramer Class II is not adequately supported by the presently available databases but notes that it could be useful for some specific groups of chemicals, for which a significant number can be assigned to Cramer Class II. It was further noted that the procedure used by EFSA for evaluation of flavouring substances differs somewhat from the general TTC approach, in that flavourings are evaluated in structurally-related groups for which some toxicity data is often available on one or more members of the group, and that the evaluations are also enhanced by prediction of likely metabolic routes.

Some comments expressed concern that the TTC relies on predictions from (Q)SARs, yet many (Q)SAR approaches have been shown to be unreliable predictors of toxicity.

These comments are based on a misunderstanding that, because the TTC approach uses structural Cramer Classes and structural alerts for genotoxicity, this is the same as relying on (Q)SARs for prediction of particular types of toxicity. The Scientific Committee working group agrees that such (Q)SARs often have poor predictive ability, except for some that are used to predict genotoxicity. In the TTC approach, structural elements of substances are used only to group chemicals into Cramer Classes or to identify the presence of well-agreed structural alerts for genotoxicity.

2.4. Comments related to exposure

Some comments questioned the advice regarding exposure estimates in that the opinion does not state there is a need to take account of exposure to same substance from all routes and sources.

This had been addressed in earlier versions of the draft opinion but it had been inadvertently omitted in the text for public consultation. The relevant text has been restored.

Some comments asked what would be the consequences when the TTC approach was applied to a substance but exposure exceeded the relevant TTC value.

It is clear from the wording in the generic scheme for the application of the TTC approach in the opinion that a non-TTC approach is then required, such as the provision of toxicity data or the use of read-across to closely structurally-related substances on which there are toxicity data.

2.5. Comments relating to applicability of the TTC values for infants and children

Some comments questioned whether the TTC values are adequately protective for infants and children, given their lower body weights and the fact that their metabolic and elimination processes are not fully mature. Other comments argued that TTC values are sufficiently conservative because the underpinning databases include studies on prenatal, early postnatal and lifetime exposures.

In the generic scheme for application of the TTC approach in EFSA's work, the TTC values in the draft opinion were already expressed in terms of body weight, rather than per person as originally proposed by Munro et al. (1996), since the latter were calculated for 60 kg adults. Values expressed on a body weight basis take account of the lower body weights of infants and children.

Scientific literature describing the maturation of different metabolic pathways and elimination processes in young infants is cited in the opinion. The Scientific Committee has also considered

whether the TTC approach could be applied to young infants under the age of 6 months, in whom not all metabolic and elimination processes are yet mature. The toxicokinetic differences between young infants and children or adults are transient and generally not more than 2- to 5-fold. The SC considered that there is capacity in the first weeks of life to metabolise and eliminate substances, particularly when exposures are low. The Scientific Committee concludes that the TTC approach can be applied to assess exposures in young infants, but in cases where the estimated exposure is in the range of the TTC value, additional consideration needs to be given under which conditions the TTC approach could be used. Additional considerations might include prediction of metabolic routes for the structure concerned and other issues such as frequency and duration of the exposure.

CONCLUSIONS:

A revised version of the opinion was produced and discussed by the Scientific Committee on 7 February 2012, during its 53rd Plenary meeting, and on 17 April, during its 54th Plenary meeting. A revised draft opinion was tabled for adoption at the 55th Scientific Committee on 22nd May 2012.

Appendix A – Organisations that submitted comments to the consultation (in alphabetical order)

ORGANISATION	COUNTRY
Agence nationale de sécurité sanitaire, de l'alimentation, de l'environnement et du travails (Anses)	FRA
BASF SE	DEU
Cancer prevention & Education Society	GRB
ClientEarth	BEL
Confederazione Nazionale Coldiretti	ITA
DSM	NLD
DuPont	USA
Danish Environmental Protection Agency	DNK
ECETOC	BEL
Fraunhofer ITEM, Hannover	DE
France Nature Environment	FRA
Federal Office of Public Health	CHE
Federal Public Service Health, Food Chain Safety and Environmental	BEL
EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA)	BEL
UK Food Standards Agency	GBR
Generation Futures	FRA
Kantonales Labor Zürich	CHE
Karolinska Institutet	SWE
HESI (ILSI Health and Environmental Sciences Institute)	USA
International Life Sciences Institute – European branch (ILSI Europe aisbl)	BEL
International Life Sciences Institute - International Food Biotechnology Committee (ILSI IFBiC)	USA
INRAN, Rome	ITA
Office Risk Assessment VWA	NLD
PAN Europe (Pesticides Action Network)	BEL
PlasticsEurope	BEL
R.I.S.K. Consultancy	BEL
Reseau Environmental Sante	FRA
RIVM - Dutch National Institute for Public Health and the Environment	NLD
Swedish Chemicals Agency	SWE
SEMAEST	FRA
Technical Universiy of Denmark, National Food Institute	DNK
TNO	NLD
Unilever	BEL
WWF European Policy Office	BEL
Xiphora Biopharma Consulting	GBR

APPENDIX B

COMMENTS SUBMITTED BY MEANS OF THE ELECTRONIC FORM ON THE EFSA WEBSITE (ORGANISED BY SECTIONS OF THE OPINION)

	Chapter of the opinion	Organisation	Comment Text
1	1. Introduction	ILSI Europe aisbl	The ILSI Europe TTC Task Force respects and highly appreciates the critical scientific evaluation conducted by the EFSA committee of all the analyses by Cramer, Gold, Cheeseman, Munro etc. that went into the final Kroes et al (2004) decision tree and other TTC schemes. The opinion draft provides an excellent summary of the analysis of the current TTC concept as conducted by the EFSA Scientific Committee. The analysis itself was detailed, and examined many of the concepts using up-to-date data where possible, as well as analysing the chemical data set of Munro et al., and the utilisation of the Cramer et al. scheme. On the whole, the conclusions reached by the committee are agreed with, and the endorsement to use the TTC for wider application in foods is welcomed. In addition, the ILSI Europe TTC Task Force would like to bring forward few suggestions and comments on details in the Opinion, which we hope that the Committee will find useful.
2	1. Introduction	ANSES	Comments from the French agency for food, environment and occupational activities (Anses). We acknowledge the comprehensiveness of the document, and the need for such a document to be published. Within the French agency for food, environment and occupational activities (Anses), this approach is used in the case of : Pesticides metabolites in groundwater Pesticides impurities when data are sparse in the databases (in conjunction with SAR/QSAR & expert judgment). Food processing aids evaluation. Except in these specific cases, this approach is rarely used per se. However, in 2005 ANSES issued a report dealing with the same topic (TTC) and with the potential application of this concept in different fields of risk assessment. It thus may be useful for completeness to also refer to this document in the SC opinion. In addition, some of our guidelines were written taking into account the TTC concept (guidelines for detergent used on FCMs and guidelines for plastic FCMs treated by ionizing radiation) as the level of requested toxicological data increases with the estimated exposure of the consumer. The document should make more specific recommendations on the application of the TTC concept regarding the situation of use and the users: who should use the TTC concept and when? Industry for in-development compounds? Petitioner for dossier submission to Efsa? National Agency involved in risk assessment for public health? We consider that TTC concept could be applied in the following situations: - Contamination crisis with a new compound for which no toxicity data is available - Priorization of substances in a list (as ESCO list on substances in non-plastic FCMs, or heat generated compounds in food)

3	1. Introduction	FEFANA	FEFANA welcomes the approach provided in the document for consumer safety. However, EFSA would create an inconsistency issue, if such an approach would not be envisaged for the target animal safety, given the wealth of knowledge now available at EFSA level.
4	1. Introduction	Generations Futures	Adoption of TTC would mean a violation of EU policy on many levels. The non-threshold policy for genotoxic carcinogens would be removed, the policy on uncertainty factors (10 x 10) changed and watered-down severely, the testing requirements for impurities and metabolites deleted, the hazard approach for CMR's and endocrine disrupting chemicals undermined, and possibly other policy in the near future like the drinking water standard and the criteria for endocrine disrupting chemicals. The TTC proposal is a completely non-science based proposal and could be easily falsified by independent data with a factor 10, 100, 1000 and up to 7500. This means use of TTC puts people at grave risk. If TTC would be applied for pesticides for instance (not yet the case but industry keeps on pushing for general use) almost all pesticides would be classified as "safe" due to the extreme high exposure dose the TTC allows. Instead of weakening risk assessment we would encourage to support a reform of risk assessment, include the latest science especially on windows of vulnerability during developing organisms, include new finding on low doses and non-linear dose-response curves and invite actively publishing scientists to help constructing a modern risk assessment methodology that prevents harm to EU citizens. TTC totally disregards new scientific findings and science in general and we recommend it should be stopped. But we are worried about the methods used by EFSA in this affair. People heavily involved in industry lobby club ILSI and promoters of the TTC-idea in publications are part of this very EFSA panel to scrutinise TTC. How can this result in a fair or objective outcome?
5	1. Introduction	RESEAU ENVIRONNEMENT SANTE	Line 395. The TTC approach does not take into account multiple exposures to the same chemical through multiple sources. Therefore exposure estimates may lead to a serious underestimation of real life exposures. This becomes even more apparent when considering that combined exposures to different substances leading to the same adverse toxic effect are not considered. Line 402: This concept is outdated and contradictory with the strategy in development to control endocrine disrupters in view of the scientific studies reporting adverse effects of certain chemicals at very low doses as well as increased risks for adverse effects later on in life following prenatal exposures. (see e.g. the Scientific Statement by the Endocrine Society: http://www.endo-society.org/journals/ScientificStatements/upload/EDC_Scientific_Statement.pdf) Line 405: This sentence is highly questionable. To use the TTC concept beyond priority setting for the risk assessment of chemicals in general cannot be scientifically justified. The use of synthetic substances in a large variety of applications requires a sound chemical safety assessment of the substance based on substance – specific toxicity data.
6	1. Introduction	DSM	Line 80 to 86 and 92 to 99: EFSA suggests to treat substances classified Cramer Class II as substances classified Cramer Class III, therefore setting a threshold more conservative for the substances classified Cramer Class II. This is done without adapting the threshold of Cramer Class III by removing the organophosphates and carbamates substances, thus making the threshold even more conservative. This suggestion is based on the fact that the databases include few substances in Cramer Class II. However, there are many flavouring substances classified as Class II, that are also evalutated as Class II by JECFA. We wonder then what the consequences of this change would be.

7	1. Introduction	DSM	Line 154 to 156: in order to apply the TTC approach to the whole population, including infants and children, EFSA proposes to convert all TTC values in mg/kg body weight. With reference to the Draft guidance on default assumptions , written by EFSA (deadline 15 September 2011), where they suggest to use an adult body weight of 70 kg instead of 60 kg, we wonder which default body weight will be applied to the TTC approach.
8	1. Introduction	INRAN, Rome	Line 402-403 A reference is needed to support the sentence "At exposures below…". Otherwise this sentence reads as a conclusion while no argumentation has yet been provided to support it. Line 404-407 In this paragraph and in other sections of the opinion the terms "initial assessment" and "priority setting" are used to describe the objective of the TTC approach. However there is no mention in the opinion of which further assessments would be performed after the initial one or of how substances with low priority (under TTC) would be dealt with, once those with high priority have been assessed. Based on figure 2 p.47 it seems that the intention of the SC is to propose the TTC as a risk assessment tool. In fact the figure clearly appears as a "decision tree" which is be used to conclude "no safety concern" (or "low probability of safety concern") when estimated exposure is under the TTC. This is also the way in which the TTC approach is currently used by the CEF Panel for flavouring substances. If the SC proposes to use the TTC approach as a "priority setting" tool (which I would support) figure 2 p. 47 should be modified and "no safety concern" should be changed into "low priority for comprehensive risk assessment".
9	1. Introduction	Confederazione Nazionale Coldiretti	Coldiretti is keen to contribute to this Pubic Consultation on the issue of TTC. Risk assessment is by its own nature a case-by case process, rooted in pragmatic considerations more than in general a-priori assumptions on the expected fate of chemicals previously not evaluated. In particular, we believe that the scope of TTC is too wide in its goal to set a base for universal toxicological principles. Even if useful in extreme situations where an immediate need of risk management is implied, we think that risk assessment has the responsibility to drive in the long term a more responsible and well managed framework for analysis in Europe, without a "one size fits all" approach. Hence, Coldiretti thinks that departures from the MOE model suggested in 2005 by EFSA, and basing assessment on animal/rodents data, should be carefully considered. Coldiretti is aware that each year thousands of new chemicals are introduced on the market both directly or indirectly (metabolites) in a wide range of food and feed related products, including flavorings, additives and pesticides, and with benefit of consumers and industry. However, this strong marketing pressure should not be detrimental of EU's core values in food safety assessment and management, first of all, the Principle of Precaution. The use of a new model of TTC should be allowed only in very limited cases, and industry to embark in further toxicological research in case of product to be launched on the market and for which there is still a lack of data. For this purpose, a more traditional "regulated product" risk assessment should be carried out. In fact, the effect of the adoption of TTC on industry as a whole is difficult to predict. Industry could be easily tempted to flow

			the market with products of unproven toxicological profile, due to virtual and abstract chemical structure specifications only. The problem in terms of public health effect, could be here the sum of the parts, not considered in the single evaluation of each chemical. This law is well known in economics and policy decision making under the name “unintended consequences”. Assuming the idea that a threshold of toxicological effect has to be surpassed in order to trigger a negative endpoint, there is a lack of information about in real life of summing up more per-se safe chemicals. Saturation and overload of cells repair capacity at high doses of chemicals/metabolites are to be explored jointly once the substances have been placed on the market. Coldiretti fears furthermore the wider use of TTC approach proposed by EFSA SC may transfer costs of toxicological assessment from industry to unknown risk on consumer. With such proposal EFSA is acting more as risk manager than risk assessor, making political choices under societal judgements (including animal testing) and allocating resources. As Ulrich Beck seminal thinking in the ”Risk Society”, we should be aware than actually on the field there is the decision: “who should carry the implicit risk, and why?” The principle of providing safe food products, according to reg. 178 General Food Law, is in charge to the producer. This is an universally applicable principle which stress the liability to test food safety in case of the will to introduce new ingredients, additives, chemicals or predictable metabolites.
10	1. Introduction	FEFANA	Lines 61 and 62: the EFSA Scientific Committee considers that the TTC approach should not apply when legislative requirements for data are required. FEFANA understands that this covers the case when product needs to follow a pre-marketing authorisation. FEFANA believes that the TTC approach could also be used for products that shall apply for a pre-marketing authorisation, when the low application dose is recommended.
11	1. Introduction	FEFANA	Lines 80-86, and 92-99: EFSA suggests to treat substances classified Cramer Class II as substances classified Cramer Class III, therefore setting a threshold more conservative for the substances classified Cramer Class II. This is done without adapting the threshold of Cramer Class III by removing the organophosphates and carbamates substances, thus making the threshold even more conservative. This suggestion is based on the fact that the databases include few substances in Cramer Class II. However, there are many flavouring substances classified as Class II which are also evaluated as Class II by JECFA. We wonder then what the consequences of this change would be.
12	1. Introduction	FEFANA	Lines 92-99: The possibility to exclude the organophosphates from CRAMER Class III and consequently increasing the CRAMER Class III TTC level should be considered.
13	1. Introduction	FEFANA	Line 144: Definition of the term "chemical space" somewhere in the document would be highly appreciated
14	1. Introduction	FEFANA	Lines 147-151: The possibility of time adjustment of TTC values for shorter than chronic exposures should be re-considered. Indeed, time adjustment of TTC levels in general was already evaluated and endorsed by other competent European bodies like EMA or in the USA by FDA. Time adjustment i.e. extrapolation form long-term to short-term is also possible within the REACH framework.
15	1. Introduction	FEFANA	Lines 147-151: The possibility of time adjustment of TTC values for shorter than chronic exposures should be re-considered. Indeed, time adjustment of TTC levels in general was already evaluated and endorsed by other competent European bodies like EMA or in the USA by FDA. Time adjustment i.e. extrapolation form long-term to short-term is also possible within the REACH framework.

16	1. Introduction	FEFANA	Lines 154-156: in order to apply the TTC approach to the whole population, including infants and children, EFSA proposes to convert all TTC values in mg/kg body weight. FEFANA would appreciate to have information on which basis the conversion will be done.
17	1. Introduction	FEFANA	Line 194: the EFSA Scientific Committee provides cases where the TTC approach could apply and link this to low exposure. FEFANA understands that the TTC approach compares the potential exposure of a compound with a threshold; hence a low exposure would be an exposure below the TTC. Therefore the reference to low exposure seems redundant. In addition, FEFANA considers that the TTC approach could be the first step of a stepwise approach for the evaluation of the dossier content.
18	1. Introduction	FEFANA	Lines 198-201: the EFSA Scientific Committee provide for cases where the TTC approach could apply in relation to products application for feed (e.g. technological additive). FEFANA considers that the risk assessment of a compound is not linked to its application (except the dose recommended). Therefore, FEFANA would support the application of the TTC approach for all additives, if the proposed recommended dose would lead to exposure below the TTC value of the compound.
19	1. Introduction	FEFANA	Lines 428-429: the EFSA Scientific Committee excludes the application of the TTC principle for the evaluation of target animal safety. FEFANA questions the rationale for this exemption. FEFANA considers it would be appropriate to promote a similar principle for target animal safety, with a view to reduce the use of animals in trials. Based on recent applications for feed additives, a wealth of knowledge on the safety of feed additives categories for target animal now exist and should be used as a basis for such approach.
20	1. Introduction	BASF SE	General comment: The document is generally an excellent presentation of the state of the art and the applicability of the TTC concept. The relevant references are cited and the applicabilities of the TTC concept in the different fields of risk assessment are nicely collected.
21	1. Introduction	BASF SE	General comment: Summary and Page 6, Table of content: It would be nice to present the updated TTC decision tree in the summary and to list the page 47 where it is first presented within the text. Currently the only hint regarding a TTC decision tree refers to the “old” Kroes tree.
22	1. Introduction	WWF European Policy Office	Line 395: …and information on human exposure, for which there is confidence that it is not an underestimate.” One of the limitations of the TTC approach is that it does not take into account multiple exposures to the same chemical through multiple sources (in addition to oral intake, e.g. through inhalation of house dust). Therefore exposure estimates may well lead to a serious underestimation of real life exposures. This becomes even more apparent when considering that combined exposures to different substances leading to the same adverse toxic effect are not considered. This is a challenge for all risk assessment approaches, but should be clearly highlighted here in this context. Line 399: The human exposure thresholds values have been developed based on data from extensive toxicological testing in animals. Still, the question

			remains about the quality of the underlying data, such as reliability, representativeness, and sensitivity of these partly very old data. It is well-known that the results of NOEL-studies depend on the design, i.e. depending on the duration, sample size and which endpoints and life stages were covered. This should be discussed more in detail, as the information becomes hidden and intransparent once only the threshold values are used. Line 402: At exposures below the generic human exposure values the probability of adverse effects on human health is considered to be very low. This concept is problematic and seems outdated in view of the scientific studies reporting adverse effects of certain chemicals at very low doses as well as increased risks for adverse effects later on in life following prenatal exposures. (see e.g. the Scientific Statement by the Endocrine Society: http://www.endo-society.org/journals/ScientificStatements/upload/EDC_Scientific_Statement.pdf) Line 405: “In this respect, the TTC approach has the potential to be used both for qualitative risk assessment and for priority setting, to enable efficient use of available resources.” This sentence is highly questionable. To use the TTC concept beyond priority setting for the risk assessment of chemicals in general cannot be scientifically justified. The use of synthetic substances in a large variety of applications requires a sound chemical safety assessment of the substance based on substance – specific toxicity data. Line 407: Its wider use would reduce the use of animals in toxicity testing. It should be considered whether this important aim can not be achieved better by other scientific approaches such as a combination of intelligent testing strategies, increased use and development of non-animal tests as well as higher acceptance of in-vitro regulation and increased use of all available data published in the scientific literature (even if not GLP).. These approaches are all well suited to provide high quality data without undermining the basis for human health and environment protection. Line 417: “Its use has also been proposed for assessment of…industrial chemicals under REACH (ECHA, 2008).” This sentence is misleading. The proposed use of TTC within REACH is limited to defined cases of exposure-based-waiving of specified tests. TCC does not replace the provision of hazard and exposure data or chemical safety assessment during registration. Moreover, the use of TTC for waiving purposes is also controversial (see 2005, Nordic Council of Ministers, 2005).
23	1. Introduction	Confederazione Nazionale Coldiretti	Risk assessment hierarchy of chemicals, when lacking epidemiological data or other human exposure data, -generally speaking and simplifying-, takes into account: in vitro data, in vivo (rodents) data, short term effects, long term effects, low doses effects, high doses effects, as well as biological pathways, kinetics, escretion and metabolic routes/fate. Only the (cumulative) presence of all of these quanti-qualitative steps lead to an ideal risk assessment. Obviously often real data are not present. Since -apart of any established toxicological profile- we are talking of undesirable substances (a), that have no cogent technological necessity (b), but that want to be placed on the market (c), to cut any kind of assessment in the name of Cramer classes allocation should be adequately considered. Differently, for substances and chemicals unexpected and not due to recent immission on the market of food /feed related products, TTC could have their initial utility waiting for a further RA. We think that deliberate and accidental release of substances/chemicals should be treated differently by the risk assessor. In fact the picture is confused by the fact that actual EFSA’s introduction considers both unpredictable and voluntary emission of substances. It is not the case, and due to market regulation, chemicals can end up accumulating in our food environment. Coldiretti stays stick to the actual risk assessment principles in Europe:

			- In case of neither carcinogenic and genotoxic substances, the No Observed Adverse Effect Level (NOAEL) criterion, and the subsequent fixation of an Acceptable Daily Intake should continue to be the norm, with the routinely use of 10*10 folds safety factors. Obviously it implies studies on animals, contrary to the assumptions made in the EFSA's TTC Document. We feel somehow misleading the reference that TTC could help avoid animal bioassays, which are considered the basis for any serious yet initial toxicological assessment. - For a more quantitative yet not virtual assessment (not cancer health effects)the Benchmark Dose is considered as a reference, and allowing for a deeper assessment considering also the dose-response relationship and the Margin of Exposure (MOE), fit to both cancerogenic and genotoxic substances. - A MOE of 10.000 if based on a Benchmark dose lower limit of 10%) is considered of low concern by EFSA previous work (2005). - Predicting toxicity from chemical structure Part of the scientific community considers easier to infer carcinogenic properties from the chemical structure than other toxicological outcomes, which however should be duly considered. (EPA, 2000:"However, as more chemicals are tested for toxicity and other end-points are identified in the future, the data base will become larger, which should permit more valid comparisons between structure and toxicity among more classes of compounds"). Coldiretti is concerned about toxic effects not directly related to carcinogenic and genotoxic properties, which can place farmers and food producers in a difficult position in front of the market and the consumers, when there is still a lack of knowledge on the issue. - In case of both carcinogenic and genotoxic substance, the Margin of Exposure approach seems better fit to real life. In fact, MOE does not make any assumption in terms of an aprioristic "safe intake" (EFSA, 2005), differently from TTC. Under MOE, for each chemical exposure the ratio of human exposure to the dose to increasing rodents cancer is calculated . The MOE for a chemical exposure is the ratio: between Carcinogenic Dose for 10% of Rodents (mg/kg/day) developing cancer/Average Human Exposure (mg/kg/day). And the obvious rationale is that rodents toxicology is well descriptive of human toxicology.
24	1. Introduction	Confederazione Nazionale Coldiretti	Summary ll. 35. 37 It should be clearly separated the use of TTC due to new analytical sensitivity from use of TTC for newly developed substances intended to be placed on the market -which should undertake as far as possible an ordinary risk assessment procedure-. Summary, ll. 58-62- It could be helpful to improve clarity of the text to better outline the boundaries of TTC application due to actual legislative framework in the EU. Summary, ll.100-103. In the conclusions of the document the SC (Recommendations for future work, ll 2065-2066)stresses also that a further refinement and better finalization of Cramer classes may be an area of future research. It should be stated clearly if EFSA intends to embark on Cramer classes re-adaptation before suggesting to the panels to using them or if EFSA SC considers it already by the time sufficient for risk assessment purposes.
25	1. Introduction	Confederazione Nazionale Coldiretti	Background as provided by EFSA ll. 297-303 Risk assessment is a pragmatic and case by case evaluation. In principle, only in case of not reproducible experimental conditions there should be "toxicological assumptions" as the TTC.

26	1. Introduction	Confederazione Nazionale Coldiretti	Background as provided by EFSA ll. 297-303 Risk assessment is a pragmatic and case by case evaluation. In principle, only in case of not reproducible experimental conditions there should be "toxicological assumptions" as the TTC.
27	1. Introduction	Confederazione Nazionale Coldiretti	Assessment –Introduction ll. 407 Risk assessment should be stricter and more conservative than benefits assessment- but the TTC approach relies on weaker principles of assessment than those used for instance in the health claims regulation. This is a matter of internal coherence for EFSA credibility, in our opinion. EFSA would not support any health claim based merely on in vitro tests or on expectations on chemical structure faith. Hence, the presentation of abstract classes of toxicological concerns needs a clarification from EFSA SC, and stands in contradiction with the rigid EFSA's standards of evaluation in other areas.
28	1. Introduction	TNO	In general we are very pleased with the draft EFSA opinion on 'Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC)" and the opportunity for a more widely application of the TTC concept in the safety evaluation of food products. We have one general comment and some specific comments which are stated in the respective sections. We hope that these comments are useful. General comment: It is noted that no attention is paid to the application of the TTC approach for the safety assessment of unidentified substances. However, the International Life Sciences Institute (ILSI) Europe has developed a step-wise approach to assess the safety of unidentified substances found upon food analysis. This work was recently published in Food and Chemical Toxicology (Koster et al, 2011). Besides, TNO (The Netherlands) has developed a framework to apply the TTC concept to complex food matrices and also published their work in Food and Chemical Toxicology (Rennen et al., 2011). We would highly appreciate it if a section could be added on the application of TTC to unidentified substances and complex matrices.
29	1. Introduction	ClientEarth	By promoting TCC as a viable tool for risk assessment and priority setting, EFSA fails to protect the interests of the public. TCC values are supposed to represent virtually safe exposure doses. They are promoted as probabilistically derived risk assessment (RA) tools which can be used to screen out chemicals for which detailed toxicological tests are required, reducing the need for animal testing and enabling resources to be used more efficiently. In biology, the chemical interactions governing development and homeostasis form very complex networks which can be exquisitely sensitive to external chemicals at certain points in their development and resistant at others. Individuals within a population may show greater or lesser sensitivity depending on their endogenous hormone levels and/or age or disease status. It is also not uncommon for chemicals and endogenous hormones to have different biological properties at different doses, and a chemical found to be safe at a relatively high exposure dose may not be safe at a lower one, particularly during sensitive stages of an organisms' development. Any exposure dose determined to be "safe" must therefore be set with great caution, and should err strongly towards the precautionary principle. Many chemicals, particularly those which act as endocrine disrupting chemicals (a type of toxicant which is entirely disregarded in the determination of TCCs), have been shown to have LOELs and NOELS much lower than their TCCs. Bisphenol A, for example has a TCC value of 150µg/kg bodyweight/day and an assumed TCC NOEL of 1.5µg/kg/day. Independent studies, however, have established a LOAEL for bisphenol A of a mere 0.025µg/kg/day (Muñoz-de-Toro et al 2005)!

30	1. Introduction	RIVM	General statements These comments have been compiled from employees at the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands. However, they should not be considered as a formal comment from RIVM / Dutch authorities. We are aware that Mrs A. Bulder, who is also an RIVM colleague, is a member of the Working group on the TTC Opinion. In order not to compromise her independency, on purpose we have not consulted her in the development of our comments. The efforts of EFSA to further strengthen the concept of TTC application in risk assessment is highly appreciated. This concept is in our view extremely helpful for the evaluation of low levels of exposure and may contribute significantly to the reduction of animal testing for (notified) substances, in case low levels of exposure are anticipated. Comments to the draft opinion (A comment to the Abstract) Pg 1, line 27-28: This sentence contains a double negation which makes it very difficult to understand the meaning of it. (Comments to the Summary) Pg 2, line 47: Maybe it is better to speak about pesticide break-down products since e.g. photo-oxidation or hydrolysis in the environment or ground water may occur without intervention of living organisms (see also line 358). In addition, to our understanding EFSA is at least exploring the use of TTC for the evaluation of pesticide metabolites in plants, which might also be mentioned here.
31	1. Introduction	SEMAEST	Une réelle consultation publique européenne nécessiterait une traduction dans toutes les langues européenne dont le Français et une accessibilité aux particuliers. Je suis contre la notion de seuil, trop dangereuse, et pour un objectif zéro. Les produits chimiques n"ont rien à faire dans la nourriture et les hommes s"en sont passé pendant des siècles. La pratique des tests actuelle paraît meilleure, à condition de communiquer pour mettre en valeur les bonnes pratiques et sanctionner les mauvaises. Il faut favoriser la culture raisonnée et tout mettre en oeuvre pour arrêter la culture intensive.
32	1. Introduction	Federal Office of Public Health	Line 415-417: It would be more precise to refer to works in which the applicability of the TTC concept in the area of contaminants in drinking water has been profoundly demonstrated as in: - Brüschweiler B.J. (2010). The TTC concept. Method of assessment of contaminants of unknown toxicity in drinking water. gwa (Gas – Wasser – Abwasser) 4:295-303 (in German). - Brüschweiler B.J. (2010). TTC-based risk assessment of tetrachlorobutadienes and pentachlorobutadienes - the in vitro genotoxic contaminants in ground and drinking water. Regulat. Toxicol. Pharmacol. 58:341-344. - Gross et al. (2010) refer to endocrine active substances in the aquatic environment. However, endocrine active substances had been previously excluded from the TTC concept and the target organisms in the aquatic environment are not primarily humans as for drinking water.

34	2. Development of the TTC concept	Technical University of DK, National Food Institute, Contaminant Research Group	We agree that a priority tool is needed for prioritizing the future evaluation of the many non evaluated substances in the field of food packaging materials (of non-plastic). However, we find that the proposed concept needs evaluation and validation on data within this field. We find that special focus should be on the principle for classification of the different substances. Evaluation and validation of the tools is needed to ensure that no false negative conclusions are made especially with the classification of carcinogens but also with other effects.
35	2. Development of the TTC concept	ClientEarth	Any probabilistic risk assessment methodology relies heavily on having access to large quantities of good quality data. Despite this, TCCs are promoted as an alternative to full toxicological testing for compounds for which scant data are available. When data are available, only those derived from OECD/GLP protocol chronic toxicity tests conducted by industry are used, excluding all data generated by studies conducted by financially independent scientists and thus creating a massive conflict of interest. Despite the development of a plethora of in vitro tests by the pharmaceutical industry and academia, and their potential utility in screening chemicals for toxic properties cheaply without the use of animals (as they are used in pharmaceutical research), only a few crude in vitro tests are used. The OECD/GLP protocol chronic toxicity tests use small groups of genetically homogeneous animals and often do not cover developmental exposure. The range of doses used is small and usually near to the concentrations known to cause acute illness. No attempt is made to determine how the chemicals may behave in combination with other chemicals present in the environment, or whether they are likely to aggravate disease states which involve perturbed hormone levels. Relatively few endpoints are studied, and the methods used are primitive. Histological examinations of tissues using a light microscope are still favoured despite being unable to give much information about the biochemical state of the tissues. Animals are never allowed to complete their life cycles, typically being euthanised shortly after birth, during young adulthood or at an age equivalent to the human age of 60, so adverse effects in old age resulting from developmental exposures or other exposures earlier in life cannot be accounted for. It is therefore impossible to say that these tests can be expected to accurately predict how a chemical is going to behave in a wild, genetically homogenous population exposed to real world doses and mixtures of chemicals.
36	2.1. Underlying principles	Swedish Chemicals Agency	General comment: Background as provided by EFSA. Line 305-335: It is refreshing to see that EFSA are ambitious, alert and quick to take new developments and new concepts into account. Initiatives such as "Toxicity testing in the 21st century" (NRC, 2007) will greatly improve risk assessments. Underlying principles: Line 434-435: While the concept that toxicity is a function of dose is valid for most substances, it must be kept in mind that great caution is needed when it comes to substances with possible endocrine disruption properties and also combination effects (cocktail) of several substances. For both of these, effects can occur in the low-dose spectra, far below NOAEL levels, and hence far below TTC.

37	2.1. Underlying principles	ClientEarth	Furthermore, data derived from quantitative structure-activity relationship studies (QSAR) studies is deemed to be adequate for determining the TCCs of chemicals for which there are few or no data. Potentially any chemical can have its structure studied and a TCC derived from these data, but this is far from an adequate basis for making regulatory decisions regarding safe exposure levels. Whilst QSAR studies have proven useful in identifying chemicals with the potential to cause certain toxic effects, the inherent complexity of the biological systems they interact with in vivo make these studies very prone to producing false positives and negatives. It is also foolish to use Cramer classes, categorisations based on an analysis carried out in 1978 (Cramer et al 1978) on the relationship between chemical structures and certain types of toxicity, in the determination of safe exposure levels. In addition to the abovementioned deficiencies of QSAR, the toxicity types used fail to cover many aspects of toxicity since they were formulated principally to identify carcinogens and exclude areas such as endocrine disruption and developmental toxicity which were unknown or much more poorly understood in 1978 than they are today! The issue of TCCs highlights an apparent cultural deficiency within organisations such as EFSA, whereby staff accept industry promoted Risk Assessment tools such as TCCs, and are thus sheltered from data generated by financially independent scientists which calls the validity of these tools into question. TCCs are neither an adequate substitute for chemical toxicity testing nor a helpful addition to it, and unless it is abandoned in favour of a more balanced, quantitative approach the true risks posed by chemicals to consumers will remain undetermined.
38	2.1. Underlying principles	RESEAU ENVIRONNEMENT SANTE	Line 434. The assumption behind TTC that toxicity is a function of the dose doesn't take in account the shift of paradigm currently at the core of a revolution in toxicology. The old principle that doses makes the poison doesn't stand alone any more. Other fundamental principles have been demonstrated such as the critical windows of exposure, e.g. during pregnancy. In addition, non-monotonic dose response curves have been described, in particular in the case of hormone disrupting chemicals. All these findings seem to be reflected very little in the current EFSA opinion. This seems like an unjustifiable omission of relevant parts of science. See for example work carried out by the Society of Toxicology: http://www.toxicology.org/ai/pub/SI10/SI10_pptox.asp Line 440: The basis for all recent reforms of chemical regulations, such as REACH, is a recognition of insufficient data and knowledge, in particular of long-term effects, of the vast majority of chemicals. Moreover, many cases such as the current controversy on Aspartame, tend to show that even food, cosmetics or pharmaceuticals are fields where not only knowledge is lacking but data are often misleading as relying too much on industry and on obsolete procedures not protective enough against conflict of interest.
39	2.1. Underlying principles	WWF European Policy Office	Line 434. The TTC concept has its origin in one of the fundamental principles of toxicology, that toxicity is a function of the dose. It is certainly true that the dose is a very important principle of toxicity. However, other fundamental principles have been demonstrated such as the critical windows of exposure, e.g. during pregnancy. In addition, non-monotonic dose response curves have been described, in particular in the case of hormone disrupting chemicals. All these findings seem to be reflected very little in the current EFSA opinion. This seems like an unjustifiable omission of relevant parts of science. See for example work carried out by the Society of Toxicology: http://www.toxicology.org/ai/pub/SI10/SI10_pptox.asp Line 440: “The TTC approach could be applied a priori to any substance. It would not usually be used to assess substances in food for which appropriate toxicity data exist or for which regulatory authorities normally require toxicity data to be submitted. However, it could also be useful for priorisation of substances for further risk assessment, e.g. in case of limited toxicological data.” This phrase supports the use of TTC beyond priority setting for the risk assessment of chemicals in general. Such a use cannot be scientifically justified. Use of synthetic substances in a large variety of applications requires a sound chemical safety assessment of the substance based on substance – specific toxicity data.

			For many substances no sufficient data on long-time toxicity data are available (see Allanou et al, 1999, Public availability of data on EU High Production Volume Chemicals, JRC) and an extension of the TTC approach would undermine the necessary substance-specific hazard assessment.
40	2.2. Derivation of human exposure threshold values…	Xiphora Biopharma Consulting	ABSTRACT Line 120-126 The referenced publication by Kroes et al is non-transparent in that the compounds in the database (730 "carcinogens") are not specified nor are TD50 values cited. I have tried to obtain the database from some of the co-authors and from ILSI who informed me that it was "not available". Given this lack of transparency, I believe that it is not tenable to consider the Kroes et al assessment as "robust"; an independent raw data audit is recommended, with cross-checking of TD50 values between the ILSI dataset and the true values taken from the CPDB. Further issues are: - Linear extrapolation of TD50 values employed by Kroes et al is considered inappropriate and overconservative particularly for nongenotoxic carcinogens. The report itself mentions serious reservations about this kind of approach on Lines 707-730. - The dataset used by Kroes et al seems to be a slightly updated version of that used by Cheeseman et al, 1999; there are serious concerns regarding the integrity and validity of this dataset in terms of clear mistakes in transcription of values from the CPDB Berkeley website (see later section for examples) and in terms of using the lowest statistically significant point-estimate of TD50 rather than employing a weight-of-evidence approach. The latter has the effect of turning many compounds that are listed in the CPDB as having "no positive test" into "carcinogens". This leads to compounds such as endosulfan and rotenone being listed as potent "carcinogens" with TD50s of 0.319 and 0.433 mg/kg/day respectively. Other examples are: parathion, 3-nitropropionic acid, azinphos methyl, fenvalerate, adipamide, 2,4-dichlorophenoxyacetic acid, isooctyl ester, diphenyl carbonate, dibutyltin acetate, hydrochlorthiazide, diphenhydramine HCl, 1-naphthalene acetic acid, p-chloroaniline, malathion, 1-chloro-2,4-dinitrobenzene, titanocene dichloride, 1-naphthalene acetamide, phenol, acetohexamide, 1,1-dichloroethane, geranyl acetate. Line 141-146 The conclusion that the "carcinogens" extracted from the CPDB are representative of the "world of chemicals" seems barely plausible. According to the Bassan report, 609 structures of known "carcinogens" have been evaluated by various methods and found to occupy a similar "chemical space" to a set of compounds randomly selected from the DSSTox dataset (presumably mostly non-carcinogenic and non-genotoxic). If this conclusion is indeed correct it suggests that attempts to distinguish noncarcinogenic from carcinogenic compounds based on an evaluation of structure is pretty much doomed to failure. It may be the case that the techniques used by Bassan et al may be insufficiently discriminating to distinguish a dataset comprising 100% "carcinogens" from a randomly selected dataset. Moreover, in the Kroes dataset it is claimed that there were 105 N-nitroso compounds and 162 aromatic amines from a total of 730 compounds, which seems hardly representative of substances likely to be present in food. For example, phenols, polyphenols and carbohydrates appear to be essentially absent. A re-evaluation of the claim of "representiveness" seems appropriate.

41	2.2. Derivation of human exposure threshold values…	Xiphora Biopharma Consulting	Line 461-473 The methodology underlying the concept that a TTC of 0.15 mcg/day is appropriate for a structurally alerting compound is considered to be fundamentally flawed and highly biased. Both Cheeseman et al and Kroes et al selected "carcinogens" from the CPDB. It must be realised that only around 50% of compounds listed in the CPDB are carcinogenic and to select only those that are carcinogenic produces an immediate source of bias. In the 2005 supplement to the CPDB there was a total of of 1485 chemicals of which 751 were considered to be carcinogenic: http://toxsci.oxfordjournals.org/content/85/2/747.full. [This number could be boosted considerably by using the lowest statistically significant TD50 point-estimate appoach of Cheesman and Kroes.] There are numerous structurally alerting noncarcingens in the current dataset that contains 1547 chemicals; at least 40 such compounds can be readily identified ranging from Michael acceptors (such as allyl chloride, trans-cinnamaldehyde), aromatic amines (such as p-anisidine, p-chloroaniline, 2,6-dimethylaniline), halo alkanes (such as n-butyl chloride and 1,1,1-trichloroethane), nitro aromatics (such as p-nitroaniline, 1-chloro-2,4-dinitrobenzene), aldehydes (such as bromoacetaldehyde, glycidaldehyde), nitroso compounds (such as N-nitrosocimetidine, nitrosomethyphenidate) and epoxides (such as d,l-diepoxybutane). Clearly, the methodology employed fails to capture non-carcinogens listed in the CPDB. Moreover, conventional structural alerts for many groups of compounds dramatically overpredict genotoxic activity (ie Ames-test positivity in relation to DNA-reactivity). For example, of the alkyl aldehydes only formaldehyde is Ames-positive; it"s also non-carcinogenic by the oral route. The alkyl chlorides are generally feeble mutagens and carcinogens, n-propyl chloride and higher being non-mutagenic/carcinogenic. Only a few carbamates are genotoxic/carcinogenic; numerous carbamate agrochemicals are neither genotoxic nor carcinogenic; alfa, beta-unsaturated aldehydes, ketones and esters are almost always Ames-negative (eg mesityl oxide), etc. So the current paradigm that requires a compound with a structural alert to be controlled at the TTC of 0.15 mcg/day is based on dramatically over-predictive structural alerts and a dataset selected from the CPDB that contains 100% carcinogens and completely ignores structurally alerting non-carcinogenic compounds. A re-evaluation of the current approach is considered appropriate, incorporating the large amount of negative data that appears to be excluded from the existing methodologies.
42	2.2. Derivation of human exposure threshold values…	INRAN, Rome	Line 445-459 There is a need to state in the main text of the opinion what the 0.15 µg/person per day threshold represents according to the SC Committee and if the SC agrees with the method used by Kroes et al (2004) to derive it, as illustrated in Annex A. In Annex A the method is illustrated by stating that "The differences between the different structural alerts was most apparent in the data for the fraction of substances within each group giving an estimated upper bound risk of cancer of greater than 1 x 10-6 when present in the diet at a concentration of 0.15 µg/person per day. This value was therefore selected as the generic TTC for substances with a structural alert for genotoxicity." A more detailed explanation would be useful. It is also mentioned in annex A (line 2646-2647) that "substances for which the risk was greater than 1 x 10-6 at an exposure of 0.15 µg/person per day were further examined (see 2.2.3 below)." Such examination would be of high interest, however there is no paragraph 2.2.3 in the opinion. It would be important to quantify the percentage of substances which fall in this case. The argument of the SC for not revisiting the database is not strong enough (line 2650 of Annex A).

43	2.2. Derivation of human exposure threshold values…	WWF European Policy Office	The TTC concept assumes that carcinogenicity is the most severe adverse effect that humans need to be protected from. In the light of increasing chronic diseases such as diabetes, cardiovascular diseases and allergies it is questionable if this assumption is still justified and appropriate. This decision is not a scientific-based judgement but rather a value decision and should clearly be stated as such.
44	2.2. Derivation of human exposure threshold values….	RESEAU ENVIRON NEMENT SANTE	Line 444 The TTC concept assumes that carcinogenicity is the most severe adverse effect that humans need to be protected from. In the light of increasing chronic diseases such as diabetes, cardiovascular diseases and allergies it is questionable if this assumption is still justified and appropriate. This decision is not a scientific-based judgement but rather a value decision and should clearly be stated as such.
45	2.3. Derivation of human exposure threshold values…	WWF European Policy Office	Line 488 ff It should be added here that Cramer et al 1978 clearly stated that their tool can give a "preliminary assessment of probable risk", necessary for prioritizing which substances need an in-depth toxicological analysis. It cannot replace a comprehensive risk assessment for substances which are intentionally used in food, feed and further products. If in specific cases thresholds for assessment of exposure situations are needed, they should be set using the lowest available data on toxicity instead of using 5th percentiles as cut-off level.
46	2.3. Derivation of human exposure threshold values…	Confederazione Nazionale Coldiretti	ll. 494-498 We strongly support the idea at ll 915 to "move the 5% percentile downwards"if the SC considers it useful and more conservative.
47	2.4. The TTC decision tree	RESEAU ENVIRON NEMENT SANTE	Line 518 – 528 it should be stated that Kroes et al (2004) also excluded endocrine disrupting chemicals from the application for TTC.
48	2.4. The TTC decision tree	WWF European Policy Office	Line 518 – 528 it should be added that Kroes et al (2004) also excluded endocrine disrupting chemicals from the application for TTC (see chapter 4.3.4). Figure 1 The assumption that chemicals can be segregated into linear versus threshold dose-response chemicals is flawed. The application of this concept determines the TTC threshold values, and the use of this approach, which is based on a presumption of safety below threshold values, is likely to result in inaccurate assessments for many chemicals. Moreover, even NOELs which are often treated as "no effect levels" may be associated with effect levels of up to 20%, as recently stated by the EC scientific committees in their draft opinion on mixture assessment

			http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scher_consultation_06_en.htm. This illustrates the problem of basing the TTC concept on these data. Often in particular long-term toxicological studies cause remarkable shifts in substance-specific “thresholds of concern”. In addition, it cannot be excluded that substances have toxicological properties not known so far. New toxicological findings and new properties are not automatically covered by the database of existing toxicological knowledge from which the present TTCs are derived. An improved understanding of the hazard properties of chemicals cannot be achieved if toxicological testing is replaced by the use of default values.
49	2.4. The TTC decision tree	BASF SE	Page 12; line 510 ff: One open question is what would be the implications of the presence of a genotoxic alert in a given substance and whether or to what extent either specific genotoxicity data and/or read across would be accepted to overrule the alert.
50	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	Studies Finding Dangerous False Negative Error of S-AR: annotated bibliography Below is a small sampling of the failures & problems of relying on a chemical’s structure can predict its toxicity—i.e. S-AR. S-AR is often reasonably accurate, but determining risks requires far higher accuracy than say 70% true positive & true negative predictability (when tested against know toxicities), a typical finding from the SA-R literature. Thus S-AR can be very valuable to rapidly screen hundreds or thousands of un-assessed chemicals (a common predicament of our petro-chemical age!). But if SA-R is to predict risks, a far higher accuracy is required to protect us. By far the largest category of chemicals for which a TTC has been proposed are the Cramer S-AR classes—potentially tens of thousands of chemicals. Thus S-AR is another failure of the reliability and safety of a TTC.

51	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	J Exp Clin Cancer Res. 2004 Mar;23(1):5-8. Chemical structure of mutagens and carcinogens and the relationship with biological activity. Benigni R. Dept. of Environment and Primary Prevention, Istituto Superiore di Sanita", Rome, Italy. Chemical carcinogenicity has been the target of numerous attempts to create predictive models alternative to the animal ones, ranging from short-term biological assays (e.g. mutagenicity tests) to theoretical models. Among the theoretical models, the application of the science of Structure-Activity Relationships (SAR) has earned special prominence. SAR has been applied both in a qualitative way (for example as simple recognition of suspected sub-structures or Structural Alerts), and in a quantitative way (Quantitative SAR, QSAR) to build mathematical models linking the physical chemical or structural properties of the molecules to the toxicological endpoints. This paper summarizes the contribution that the two approaches can provide in different situations. It concludes that the study of the structure of the chemicals generates predictions with limited reliability for the individual chemicals, however it has been demonstrated to be an extremely powerful tool for priority setting relative to large samples of chemicals. Mutat Res. 1998 Oct 12;421(1):93-107. Profiles of chemically-induced tumors in rodents: quantitative relationships. Benigni R, Pino A. Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanita, Viale Regina Elena 299-00161, Rome, Italy. rbenigni@net.iss.it The rodent carcinogenicity bioassay has been used for several decades for evaluating hundreds of chemicals, with the two aims of better understanding the etiologies of cancer, and of assessing the hazard posed by environmental and industrial chemicals. This has generated an enormous wealth of data and information on the phenomenon of chemical carcinogenicity. However, this information cannot be appreciated easily, since too many details may obscure the general trends present in the data; on the contrary, the use of computerized data analysis techniques suitable for the exploration of large databases makes its investigation much more fruitful, and its results more reliable. For this work, we collected a database of 536 rodent carcinogens, and we investigated the profiles of tumors (target organs) induced in the four experimental systems which are usually employed (rat and mouse, male and female). The analysis was performed with an Artificial Neural Network called Kohonen Self-Organizing Map, which is a computer-intensive method aimed at making the relevant information emerge automatically from the data itself. The analysis generated a global view, as well as a quantitative measure of the associations among the individual tumor types, and among the tumor profiles induced by the chemicals. In the complex interplay between the organ and species specificity of tumor induction, the species specificity generally overcame organ specificity, except for a few tumors (namely Lymphatic System, Brain, Forestomach, Stomach and Thyroid Gland). Moreover, the species specificity was remarkably stronger than the trans-species sex specificity. For three chemical classes (Aromatic Amines, Electrophilic/Alkylating Agents, Nitroarenes) most represented in the database, we investigated the hypothesis that a single mechanism of interaction with DNA would produce one, or a few very similar tumor profiles. Our analysis pointed out that no obvious association exists between chemical/mode of action class, and tumor profile. On the contrary, none of these classes induces a single tumor or pattern of tumors, but rather it appears that each class produces tumors at a wide range of sites. This suggests that an important determinant of the differences in tumor profile are the events that surround the ultimate mechanism of interaction with DNA. SAR QSAR Environ Res. 2010 Jan 1;21(1):57-75. Counter propagation artificial neural network categorical models for prediction of carcinogenicity for non-congeneric chemicals. Fjodorova N, Vracko M, Jezierska A, Novic M. National Institute of Chemistry, Ljubljana, Slovenia. natalja.fjodorova@ki.si One of the main goals of the new chemical regulation REACH (Registration, Evaluation and Authorization of Chemicals) is to fill the gaps on the toxicological properties of chemicals that affect human health. Carcinogenicity is one of the endpoints under consideration. The information obtained from (quantitative) structure-activity relationship ((Q)SAR) models is accepted as an alternative solution to avoid expensive and time-consuming animal tests. The reported results were obtained within the framework of the European project "Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR)". In this article, we demonstrate intermediate results for counter propagation artificial neural network (CP ANN) models for the prediction category of the carcinogenic potency using two-dimensional (2D) descriptors from different software programs. A

			total of 805 non-congeneric chemicals were extracted from the Carcinogenic Potency Database (CPDBAS). The resulting models had prediction accuracies for internal (training) and external (test) sets as high as 91-93% and 68-70%, respectively. The sensitivity and specificity of the test set were 69-73 and 63-72% correspondingly. High specificity [true negatives] is critical in models for regulatory use that are aimed at ensuring public safety. Thus, the errors that give rise to false negatives are much more relevant. We discuss how we can increase the number of correctly predicted carcinogens using the correlation between the threshold and the values of the sensitivity and specificity. Mutagenesis. 2010 Jul;25(4):335-41. Structural analysis and predictive value of the rodent in vivo micronucleus assay results. Benigni R, Bossa C, Worth A. Department of Environment and Health, Istituto Superiore di Sanita", Rome, Italy. romualdo.benigni@iss.it In vivo genotoxicity studies-shortly followed by carcinogenicity-are posing high demand for test-related recourses in terms of animal lives and resources. Among those, the micronucleus test in rodents is the most widely used as a follow-up to positive in vitro mutagenicity results; therefore, the development and extensive use of estimation techniques based on the concept of Structure-Activity Relationships-such as (Quantitative) Structure-Activity Relationships, read-across and grouping of chemicals-might have a huge saving potential for this end point. In this paper, we present a newly derived compilation of Structural Alerts for the rodent in vivo micronucleus assay, thus providing a coarse-grain filter for preliminary screening of potentially in vivo mutagens. The compilation has been implemented as computerized rule of the expert system Toxtree and is freely available: http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE. In addition, analyses on the performance of the micronucleus assay as pre-screening tool for carcinogenesis indicate that this assay is prone to give false-negative predictions and point to the need of improving the in vivo component of the present testing schemes.
52	3. The Cramer classification scheme and its software implementation	PlasticsEurope	Comments by PlasticsEurope to the EFSA public consultation on the draft opinion of the EFSA Scientific Committee on the use and benefit of the concept of Threshold of Toxicological Concern (TTC) to assess potential human health risks associated to food consumption The comprehensive review that the Scientific Committee members conducted on the TTC concept demonstrates its usefulness as a conservative and robust tool for risk assessment pertaining to food safety. As reported in the draft opinion, there are many synthetic and naturally occurring substances, along with their respective breakdown products and impurities which are present in food and feed. As progress in analytical sensitivity has resulted in the detection of an increasing number of substances, it becomes more and more clear that many of these substances lack robust toxicological datasets. The use of TTC will reduce additional animal use in toxicity testing and corresponding time and cost for substances that will be identified as not posing a risk to human health. Additionally, the TTC concept represents a scientific sound tool for assessing potential risk to human health by identifying substances for which further toxicological information is warranted. PlasticsEurope fully supports the use of the TTC approach for risk assessment according to article 19 of Regulation (EU) No 10/2011 for food contact materials. But one proposal of the EFSA opinion merits closer attention. The envisaged allocation of the Cramer class III substances limit to class II substances would introduce a distortion with the TTC approach applied in the US, with the consequences for global companies selling the same product on both sides of the Atlantic (a risk assessment performed on the same product could lead to different conclusions in Europe and the US). The EFSA opinion highlights that the TTC value derived for Cramer Class II substances is based on toxicological data for a limited number of substances. PlasticsEurope proposes to evaluate further whether there are additional substances with toxicological data in Cramer Class II that could make the toxicological database for this Cramer class statistically more robust and support the current TTC. This would avoid the mentioned distortion.

53	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	S/AR-based method of creating a TTC vastly expands the number of chemicals potentially subject to a TTC, as any chemical can have its structure studied. It is true that correlations between chemicals' structure and their types & potency of toxicity can be very informative. For example, S-AR has multiplied by 25 the number of chemicals worth initial screening for reproductive toxicity in the EU!/1/ But there is a massive published literature showing the unreliability of assuming S-AR, for several reasons: in short, life is too varied, subtle and vulnerable for such massive predictions to be reliable enough to use to set a safe exposure level. Even if S-AR correlations are high (few false positives), they simultaneously provide too many false negatives (e.g. 30%. Such a high failure rate in predicting risks is not desirable in RA! /1/ G.E. Jensen, J.R. Niemelä, E.B. Wedebye & N.G. Nikolov. 2008 'QSAR models for reproductive toxicity and endocrine disruption in regulatory use – a preliminary investigation'. SAR & QSAR in Environ'l Res:19(7-8):631-41.

54	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	SAR QSAR Environ Res. 2003 Aug;14(4):285-316. (Q)SARs: gatekeepers against risk on chemicals? Hulzebos EM, Posthumus R. National Institute of Public Health and Environment, RIVM, Anthonie van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. etje.hulzebos@rivm.nl ECOSAR and DEREKfW predictions for the (eco)toxicological effects of circa 70 substances were compared with experimental data for risk assessment purposes. These and other (quantitative) structure-activity relationships ((Q)SARs) programs will play an important role in future chemical policies, such as in the European Union and The Netherlands, to reduce animal testing and costs and to speed up the number of risk assessments for hazardous chemicals. The two programs, ECOSAR and DEREKfW, were selected because they are easy to use and transparent in their predictions. They predict to which chemical class a substance belongs and also predict some (eco)toxicological properties. ECOSAR categorised 87% of the chemicals correctly in chemical classes. With regard to predicting ecotoxicity, criteria were drawn up for the reliability of the QSARs provided by ECOSAR. Application of these criteria had the result that half of the regression lines from ECOSAR were considered unreliable beforehand. It turned out, however, that the "unreliable" regression lines predicted similar accurately as the "reliable" lines, although much less chemicals were available for validating the "unreliable" QSARs. The overall accurate prediction of toxicity by ECOSAR was 67%. DEREKfW categorised 90% of the chemicals correctly in chemical classes, while 10% of the structural fragments needed a more detailed description. The accuracy of prediction was around 60% for sensitisation, 75% for genotoxicity and carcinogenicity for a limited number of chemicals. Irritation and reproductive toxicity were predicted poorly. Finally, it should be stressed that regulators and industries need to agree on the acceptability criteria relating to false negative and false positive (Q)SAR predictions. This to prevent unnecessary animal testing when regulators do not sufficiently rely on (Q)SAR predictions or to prevent too much faith in (Q)SAR predictions which will then may cause an insufficient protection of man and the environment. Therefore, if the regulatory trend is that (Q)SARs have to be applied more and more systematically in the risk assessment process, their validity and the available tools have to be explored further. SAR and QSAR in Environmental Research Volume 22, Issue 1-2, 2011 pages 89-106 Special Issue: 14th International Workshop on Quantitative Structure-Activity Relationships in Environmental and Health Sciences (QSAR2010) - Part 2 Structural alerts for estimating the carcinogenicity of pesticides and biocides J. Devillersa*, E. Mombellib & R. Samseràc a CTIS, Rillieux La Pape, France b Unité Modèles pour l"Ecotoxicologie et la Toxicologie (METO), INERIS, Verneuil en Halatte, France c Département des produits réglementés (DPR), ANSES, Maisons-Alfort, France More than 20 years ago, Ashby and Tennant showed the interest of structural alerts for the prediction of the carcinogenicity of chemicals. These structural alerts are functional groups or structural features of various sizes that are linked to the level of carcinogenicity of chemicals. Since this pioneering work it has been possible to refine the alerts over time, as more experimental results have become available and additional mechanistic insights have been gained. To date, one of the most advanced lists of structural alerts for evaluating the carcinogenic potential of chemicals is the list proposed by Benigni and Bossa and that is implemented as a rule-based system in Toxtree and in the OECD QSAR Application Toolbox. In order/ (continues with comment #55)

55	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	…. (continues from comment #54) to gain insight into the applicability of this system to the detection of potential carcinogens we screened about 200 pesticides and biocides showing a high structural diversity. Prediction results were compared with experimental data retrieved from an extensive bibliographical review. The prediction correctness was only equal to 60.14% [i.e. total of true pos + true neg.?]. Attempts were made to analyse the sources of mispredictions. SAR and QSAR in Environmental Research Volume 21, Issue 1-2 pages 21-35, 2010 Integrating background knowledge from internet databases into predictive toxicology models M. Edelsteina, F. Buchwalda, L. Richtera & S. Kramera* a Institut für Informatik I12, Technische Universität München, München, Germany While data integration for data analysis has been investigated extensively in biological applications, it has not yet been so much the focus in computational chemistry and quantitative structure–activity relationship (QSAR) research. With the availability and growing number of chemical databases on the web, such data integration efforts become an intriguing possibility (and, in fact, a necessity). In this paper, we take a first step towards the following vision and scenario for predictive toxicology applications. Given a new structure to be predicted, the first step would be to gather (integrate) all relevant information from internet databases for the structure itself, and all structures with available information for the endpoint of interest. In a second step, the collected information is combined statistically into a prediction of the new structure. We simulate this scenario with three endpoints (data sets) from the DSSTox database and collect information from three public chemical databases: PubChem, ChemBank and Sigma-Aldrich. In the experiments, we investigate whether the addition of background knowledge from the three databases can improve predictive performance (over using chemical structure alone) in a statistically significant way. For this purpose, we define groups of features (belonging together from an application point of view) from the three databases, and perform a variant of forward selection to include these feature groups in a prediction model. Our experiments show that the integration of background knowledge from internet databases can significantly improve prediction performance, especially for regression tasks. = = = = = = [end of the smaple of literature procing SAR"s have too high false negative rate; and the need of my comments to EFSA on its consideration of the TTC.

56	3. The Cramer classification scheme and its software implementation	PlasticsEurope	Comments by PlasticsEurope to the EFSA public consultation on the draft opinion of the EFSA Scientific Committee on the use and benefit of the concept of Threshold of Toxicological Concern (TTC) to assess potential human health risks associated to food consumption The comprehensive review that the Scientific Committee members conducted on the TTC concept demonstrates its usefulness as a conservative and robust tool for risk assessment pertaining to food safety. As reported in the draft opinion, there are many synthetic and naturally occurring substances, along with their respective breakdown products and impurities which are present in food and feed. As progress in analytical sensitivity has resulted in the detection of an increasing number of substances, it becomes more and more clear that many of these substances lack robust toxicological datasets. The use of TTC will reduce additional animal use in toxicity testing and corresponding time and cost for substances that will be identified as not posing a risk to human health. Additionally, the TTC concept represents a scientific sound tool for assessing potential risk to human health by identifying substances for which further toxicological information is warranted. PlasticsEurope fully supports the use of the TTC approach for risk assessment according to article 19 of Regulation (EU) No 10/2011 for food contact materials. But one proposal of the EFSA opinion merits closer attention. The envisaged allocation of the Cramer class III substances limit to class II substances would introduce a distortion with the TTC approach applied in the US, with the consequences for global companies selling the same product on both sides of the Atlantic (a risk assessment performed on the same product could lead to different conclusions in Europe and the US). The EFSA opinion highlights that the TTC value derived for Cramer Class II substances is based on toxicological data for a limited number of substances. PlasticsEurope proposes to evaluate further whether there are additional substances with toxicological data in Cramer Class II that could make the toxicological database for this Cramer class statistically more robust and support the current TTC. This would avoid the mentioned distortion.

57	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	Mutation Research/Genetic Toxicology Volume 371, Issues 1-2, 4 November 1996, Pages 29-46 doi:10.1016/S0165-1218(96)90092-0 QSARS of mutagens and carcinogens: Two case studies illustrating problems in the construction of models for noncongeneric chemicals*1 Romualdo Benignia, and Ann M. Richardb a Laboratory of Comparative Toxicology and EcoToxicology, Istituto Superiore di Sanitá, Viale Regina Elena 299, 00161, Rome, Italy b Environmental Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA There is a strong motivation to develop QSAR models for toxicity prediction for use in screening, for setting testing priorities, and for reducing reliance on animal testing. Decisions must be made daily by toxicologists in governments and industry to direct limited testing resources to the most urgent public health problems, and to direct the types of chemical synthesis and product development efforts undertaken. This need has motivated attempts to construct general QSAR models (e.g., for rodent carcinogenicity), not tailored to congeneric series of chemicals. These various attempts have provided interesting and important scientific evidence; however, they have also shared a limited overall performance. The goal of this paper is to illustrate, by two unrelated actual examples of QSARs for mutagens and carcinogens, some fundamental problems relative to the application of general QSAR approaches to noncongeneric chemicals. Both examples consider data sets that are noncongeneric in a chemical structure and mechanism of action sense: in the first case, a mean mutagenic potency defined as an average over multiple genetic toxicity endpoints, and, in the second case, the NTP two-sexes, two species rodent carcinogenicity bioassay results for 280 carcinogens and noncarcinogens. The problems encountered with the QSAR analyses of these two cases indicate that a successful approach to the problem of QSAR modeling of noncongeneric data will need to consider the multidimensional nature of the problem in both a chemical and a biological sense. Since different chemical classes represent largely independent action mechanisms, some means for extracting local QSARs for constituent classes will be necessary. Alternatively, a general QSAR derived for a noncongeneric data set will need to be scrutinized and decomposed along chemical class lines in order establish boundaries for application and confidence levels for prediction. J Chem Inf Model. 2008 May;48(5):971-80. Predictivity of QSAR. Benigni R, Bossa C. Environment and Health Department, Istituto Superiore di Sanita", Viale Regina Elena 299, 00161 Rome, Italy. rbenigni@iss.it A range of good quality, local QSARs for mutagenicity and carcinogenicity have been assessed and challenged for their predictivity in respect to real external test sets (i.e., chemicals never considered by the authors while developing their models). The QSARs for potency (applicable only to toxic chemicals) generated predictions 30-70% correct [true positives], whereas the QSARs for discriminating between active and inactive chemicals [true negatives?] were 70-100% correct in their external predictions: thus the latter can be used with good reliability for applicative purposes. On the other hand internal, statistical validation methods, which are often assumed to be good diagnostics for predictivity [true positives], did not correlate well with the predictivity of the QSARs when challenged in external prediction tests. Nonlocal models for noncongeneric chemicals were considered as well, pointing to the critical role of an adequate definition of the applicability domain.

58	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	Mutagenesis. 2010 Jul;25(4):335-41. Structural analysis and predictive value of the rodent in vivo micronucleus assay results. Benigni R, Bossa C, Worth A.Department of Environment and Health, Istituto Superiore di Sanita", Rome, Italy. In vivo genotoxicity studies-shortly followed by carcinogenicity-are posing high demand for test-related recourses in terms of animal lives and resources. Among those, the micronucleus test in rodents is the most widely used as a follow-up to positive in vitro mutagenicity results; therefore, the development and extensive use of estimation techniques based on the concept of Structure-Activity Relationships-such as (Quantitative) Structure-Activity Relationships, read-across and grouping of chemicals-might have a huge saving potential for this end point. In this paper, we present a newly derived compilation of Structural Alerts for the rodent in vivo micronucleus assay, thus providing a coarse-grain filter for preliminary screening of potentially in vivo mutagens. The compilation has been implemented as computerized rule of the expert system Toxtree and is freely available: http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE. In addition, analyses on the performance of the micronucleus assay as pre-screening tool for carcinogenesis indicate that this assay is prone to give false-negative predictions and point to the need of improving the in vivo component of the present testing schemes. J Chem Inf Model. 2005 Nov-Dec;45(6):1864-73. Prediction of the rodent carcinogenicity of 60 pesticides by the DEREKfW expert system. Crettaz P, Benigni R. Swiss Federal Office of Public Health, 3003 Bern, Switzerland. The two-year rodent bioassay represents the golden standard for evaluating the carcinogenicity of chemicals. Because of practical and ethical reasons, alternative approaches have been investigated for many years. Among these approaches, the (quantitative) structure-activity relationships [(Q)SARs] offer promising perspectives for quickly screening a large number of chemicals. To increase the acceptance of (Q)SARs among the regulators, their predictive power needs to be scientifically validated. In this article, we tested the capacity of the DEREKfW expert system to qualitatively predict the rodent carcinogenicity and the genotoxic potential of 60 pesticides recently registered in Switzerland. The percentage of false negatives was found to be 31% for carcinogenicity. The associated sensitivity[=true negatives] of 69% indicates that most [BARELY!!] of the pesticides with positive rodent bioassay results were detected by DEREKfW. On the other hand, the low specificity[=true positives] of 47% [i.e. 53% false positives] indicates that many pesticides may be flagged as carcinogenic while rodent bioassays would not confirm this potential. This may lead to unnecessary testing or the unnecessary restriction of a chemical [along with c. 1/3rd being sold though they are carcinogenic!].

59	3. The Cramer classification scheme and its software implementation	R.I.S.K. Consultancy	J Exp Clin Cancer Res. 2004 Mar;23(1):5-8. Chemical structure of mutagens and carcinogens and the relationship with biological activity. Benigni R. Dept. of Environment and Primary Prevention, Istituto Superiore di Sanita", Rome, Italy. Chemical carcinogenicity has been the target of numerous attempts to create predictive models alternative to the animal ones, ranging from short-term biological assays (e.g. mutagenicity tests) to theoretical models. Among the theoretical models, the application of the science of Structure-Activity Relationships (SAR) has earned special prominence. SAR has been applied both in a qualitative way (for example as simple recognition of suspected sub-structures or Structural Alerts), and in a quantitative way (Quantitative SAR, QSAR) to build mathematical models linking the physical chemical or structural properties of the molecules to the toxicological endpoints. This paper summarizes the contribution that the two approaches can provide in different situations. It concludes that the study of the structure of the chemicals generates predictions with limited reliability for the individual chemicals, however it has been demonstrated to be an extremely powerful tool for priority setting relative to large samples of chemicals. Mutat Res. 1998 Oct 12;421(1):93-107. Profiles of chemically-induced tumors in rodents: quantitative relationships. Benigni R, Pino A. Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanita, Viale Regina Elena 299-00161, Rome, Italy. rbenigni@net.iss.it The rodent carcinogenicity bioassay has been used for several decades for evaluating hundreds of chemicals, with the two aims of better understanding the etiologies of cancer, and of assessing the hazard posed by environmental and industrial chemicals. This has generated an enormous wealth of data and information on the phenomenon of chemical carcinogenicity. However, this information cannot be appreciated easily, since too many details may obscure the general trends present in the data; on the contrary, the use of computerized data analysis techniques suitable for the exploration of large databases makes its investigation much more fruitful, and its results more reliable. For this work, we collected a database of 536 rodent carcinogens, and we investigated the profiles of tumors (target organs) induced in the four experimental systems which are usually employed (rat and mouse, male and female). The analysis was performed with an Artificial Neural Network called Kohonen Self-Organizing Map, which is a computer-intensive method aimed at making the relevant information emerge automatically from the data itself. The analysis generated a global view, as well as a quantitative measure of the associations among the individual tumor types, and among the tumor profiles induced by the chemicals. In the complex interplay between the organ and species specificity of tumor induction, the species specificity generally overcame organ specificity, except for a few tumors (namely Lymphatic System, Brain, Forestomach, Stomach and Thyroid Gland). Moreover, the species specificity was remarkably stronger than the trans-species sex specificity. For three chemical classes (Aromatic Amines, Electrophilic/Alkylating Agents, Nitroarenes) most represented in the database, we investigated the hypothesis that a single mechanism of interaction with DNA would produce one, or a few very similar tumor profiles. Our analysis pointed out that no obvious association exists between chemical/mode of action class, and tumor profile. On the contrary, none of these classes induces a single tumor or pattern of tumors, but rather it appears that each class produces tumors at a wide range of sites. This suggests that an important determinant of the differences in tumor profile are the events that surround the ultimate mechanism of interaction with DNA. SAR QSAR Environ Res. 2010 Jan 1;21(1):57-75. Counter propagation artificial neural network categorical models for prediction of carcinogenicity for non-congeneric chemicals. Fjodorova N, Vracko M, Jezierska A, Novic M. National Institute of Chemistry, Ljubljana, Slovenia. natalja.fjodorova@ki.si One of the main goals of the new chemical regulation REACH (Registration, Evaluation and Authorization of Chemicals) is to fill the gaps on the toxicological properties of chemicals that affect human health. Carcinogenicity is one of the endpoints under consideration. The information obtained from (quantitative) structure-activity relationship ((Q)SAR) models is accepted as an alternative solution to avoid expensive and time-consuming animal tests. The reported results were obtained within the framework of the European project "Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR)". In this article, we demonstrate intermediate results for counter propagation artificial neural network (CP ANN) models for the prediction category of the carcinogenic potency using two-dimensional (2D) descriptors from different software programs. A

			total of 805 non-congeneric chemicals were extracted from the Carcinogenic Potency Database (CPDBAS). The resulting models had prediction accuracies for internal (training) and external (test) sets as high as 91-93% and 68-70%, respectively. The sensitivity and specificity of the test set were 69-73 and 63-72% correspondingly. High specificity [true negatives] is critical in models for regulatory use that are aimed at ensuring public safety. Thus, the errors that give rise to false negatives are much more relevant. We discuss how we can increase the number of correctly predicted carcinogens using the correlation between the threshold and the values of the sensitivity and specificity. Mutagenesis. 2010 Jul;25(4):335-41. Structural analysis and predictive value of the rodent in vivo micronucleus assay results. Benigni R, Bossa C, Worth A. Department of Environment and Health, Istituto Superiore di Sanita", Rome, Italy. romualdo.benigni@iss.it In vivo genotoxicity studies-shortly followed by carcinogenicity-are posing high demand for test-related recourses in terms of animal lives and resources. Among those, the micronucleus test in rodents is the most widely used as a follow-up to positive in vitro mutagenicity results; therefore, the development and extensive use of estimation techniques based on the concept of Structure-Activity Relationships-such as (Quantitative) Structure-Activity Relationships, read-across and grouping of chemicals-might have a huge saving potential for this end point. In this paper, we present a newly derived compilation of Structural Alerts for the rodent in vivo micronucleus assay, thus providing a coarse-grain filter for preliminary screening of potentially in vivo mutagens. The compilation has been implemented as computerized rule of the expert system Toxtree and is freely available: http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE. In addition, analyses on the performance of the micronucleus assay as pre-screening tool for carcinogenesis indicate that this assay is prone to give false-negative predictions and point to the need of improving the in vivo component of the present testing schemes.

60	3.1. Development of the Cramer classification scheme	Fraunhofer ITEM, Hannover	In the draft opinion document of EFSA it is mentioned that the "majority of substances in the Munro DB would fall into either Cramer class I or Class III, rather than Class II" (page 16). This was also confirmed by recent researches of other TTC working groups (Escher et al al. 2008, 2010, Kalkhof et al., 2011, Tluczkiewicz et al., 2011). As only few chemicals are assigned to moderate toxic Cramer class II, this class remains underrepresented and derivation of a significant threshold is not possible. EFSA also refers to "the overlap in the range of magnitudes of the NOELs between the three structural classes" (page 16) but concluded that "consideration should be given to treating substances that would be classified in Cramer class II under the Cramer decision tree as if they were Cramer class III substances" (page 44). This can not be confirmed in analyses of the Munro DB and the RepDose DB by Fraunhofer ITEM for inhalation exposure (Escher et al. 2008, 2010) and for oral exposure (Tluczkiewicz et al 2011). Both analyses revealed that the NOEL values are distributed over several orders of magnitude in Cramer classes 1, 2, and 3 resulting in a considerable overlap between the distribution curves for the three classes. But recently a strategy for better discrimination of Cramer classes was developed by Fraunhofer ITEM (Tluczkiewicz et al. 2011) so that we don not think that it is necessary to discard Cramer class II by adopting the TTC value for Cramer class III for all Cramer class II chemicals, like it is proposed in the conclusion of the EFSA report (page 44), but to further refine the Cramer decision tree. In the first step of the refinement two structural groups were reassigned to the appropriate Cramer class according to their observed toxicological potency in in vivo studies. This resulted in a better discrimination of Cramer classes 1 and 3 and increased the amount of chemicals in Cramer class II from n=12 to n=67 (Tluczkiewicz et al. 2011). Escher, S. E., Bitsch, A., Batke, M., Weiss, S., Simetska, N., Melber, C., Mangelsdorf, I., 2008. The TTC–concept for industrial chemicals. Are inhalation thresholds needed? EUROTOX 2008 Conference, Rhodes, Greece. Escher, S. E., Tluczkiewicz, I., Batke, M., Bitsch, A., Melber, C., Kroese, E. D., Buist, H. E., Mangelsdorf, I., 2010. Evaluation of inhalation TTC values with the database RepDose. Regul. Toxicol. Pharmacol. 58(2), 259-274. Kalkhof, H., Herzler, M., Stahlmann, R., Gundert-Remy, U., 2011. Thresholds of toxicological concern values for non-genotoxic effects in industrial chemicals: re-evaluation of the Cramer classification. Arch. Toxicol.2011 Jul. 7. Tluczkiewicz, I., Buist, H. E., Martin, M. T., Mangelsdorf, I. and Escher, S.E., 2011. Improvement of the Cramer classification for oral exposure using the database TTC RepDose – a strategy description. Submitted to Regul. Toxicol. Pharmacol.
61	3.1. Development of the Cramer classification scheme	Toxicologo	3. El sistema de clasificación de Cramer 3.1desarrollo del esquema de clasificación de Clamer uno e los elementos de calidad de los datos es la declaración de que fueron obtenidos utilizando procedimientos armonizados como base para la integración de los resultados de la diferentes fuentes. agradecería un comentario al respecto

62	3.1. Development of the Cramer classification scheme	RIVM	Section 3.1 Pg 15, line 590: It is stated that natural occurrence is taken into consideration. In the area of flavourings this affects quite some substance which based on their structure should be allocated in e.g. Class III but which were classified into Class II, only based on natural occurrence (often with limited underpinning). Of course this is an unwanted situation as natural occurrence in itself has nothing to do with toxicity. Next to the extremely small study-population to underpin the TTC for Class II, this is a further argument to delete the use of Class II. It would be beneficial for the evaluation of substances to redraft the Cramer decision tree to expel this criterion. If then a number of common components of food would be too severely classified, maybe other questions related to chemical structure should be included to cover this.
63	3.2. Computer-based implementation of TTC-relevant decision trees	DuPont	Lines 619-620 This appears to be an erroneous citation - Toxtree was not commissioned following a recommendation of a JRC-ECB workshop. The citation provided is Saliner et al, 2005 though this is not even listed in the references. Toxtree v1.0 was available for release in October 2005 and only relevant workshop on TTC and chemical similarity was held in November 2005. Whilst Toxtree for Cramer was commissioned following the successful award of funding for an internal ECB-JRC research project, perhaps a more appropriate citation to use would be: Patlewicz G, Gallegos Saliner A, Pavan M, Worth A, Benigni R, Aptula A, Bassan A, Bossa C, Falk-Filipsson A, Gillet V, Jeliazkova N, McDougal A, Mestres J, Munro I, Netzeva T, Safford B, Simon-Hettich B, Tsakovska I, Wallén M & Yang C (2007). Chemical Similarity and Threshold of Toxicological Concern (TTC) Approaches. Report of an ECB Workshop held in Ispra, November 2005. EUR 22657 EN.
64	3.2. Computer-based implementation of TTC-relevant decision trees	DuPont	Line 627 Worth citing the appropriate version of Toxtree that was evaluated in said paper particularly given the current version is v2.1 and has morphed from a platform for the Cramer scheme alone to many other rulebase modules.
65	3.2. Computer-based implementation of TTC-relevant decision trees	DuPont	Line 646 states that the Extended Cramer tree does not appear to be widely used and cites Chapter 3.3 yet there is no apparent discussion of the use or not of Cramer or the Extended Cramer here.
66	3.2. Computer-based implementation of TTC-relevant decision trees	Confederazione Nazionale Coldiretti	648-651 line The main problem with a qualitative decision tree is that lack of data for newly developed / recently marketed substances or secondary unexpected metabolites, which allows considerations for chemical structure only.

67	4. EFSA's consideration of the human exposure threshold values	R.I.S.K. Consultancy	Another important de-regulatory use of the TTC is revealed in the EFSA document's claim (lines 419-20) that a sister EC agency EChA (overseeing the chemicals law REACh) already uses the TTC. But what REACh actually allows is to waive toxicity tests when exposure is shown to be very low, non-existent, or adequately controlled; and REACh sets no "safe dose" to waive toxicity studies. Because EFSA strenuously emphasizes that very reliable exposure data is needed for TTC to be protective, these arguments strongly indicate that EFSA believes it is unnecessary to test the potency of toxic chemicals, allowing widespread use of the TTC…as long as exposure is sufficiently estimated as REACh already requires. Corroboration of EFSA's intent to expand use of the TTC comes from their emphasis of a published proposal to compare the exposure levels to EDC/reproductive-developmental toxics to the TTCs and if the estimated exposure level is below the TTC, waive these critically-needed tests. However, exposure estimates have always been part of a RA, but (critically) are paired with trying to test the potency of the chemical.
68	4. EFSA's consideration of the human exposure threshold values	R.I.S.K. Consultancy	PART OR FULL (in vivo) FALSIFICATIONS of the 10% Most-Potent NOELs Used to set a TTC (a random-few) Some conversions to common units, to facilitate comparison. In-vivo lo-dose results in red. compare these two columns: to this column: TTC category Randomly chosen from list of chemicals that validated a TTC : TTC:[1] TTC's assumed NOEL[2] Chemical-specific NOEL[3] Most Toxic NOEL in Independent Literature[4] Factor lower than TTC's "average" NOEL Factor lower than chem's specific NOEL Cramer Class I Styrene 30 ug/kd bw d- 3000 ug/kg bw d- 12,000 ug/kg bw d- 10.4 ug/L[5] 288 X 1,154 X Cramer Class III Dieldrin (insecticide) 1.5 ug/kg bw d- 150 ug/kg bw d- 5 ug/kg bw d- 38.1 ng [6] 0.06 ug/L[7] 2.4 ug/L[8] 381 pg[9] ~ 1,000 X 2,500 X 63 X ~ 5,000 X 83 X

			2 X ~ 150,000 X Cypermethrin (pyrethroid insect) 500 ug/kg bw d- 1.71 mg/kg brain[10] 0.0000002 ug/L[11] Not a NOEL 2,500,000,000 X Not a NOEL 750,000,000 X Diquat (herbicide) 190 ug/kg bw d- 100 ug/kg bw d-[12] 11 ug/cornea[13] 1½ X 14 X 2 X 17 X AChE-inhibiting Insecticides Diazinon Diazinon Diisopropylfluorophosphate (OP) Chlorpyrifos 0.3 ug/kg bw d- 30 ug/kg bw d- n/a 0.0005 ug/L[14] 0.00007 ug/L[15] 10 ug/kg bw d-[16] 10 ug/kg bw d-[17] 60,000 X 428,571 X 3 X 3 X n/a n/a n/a n/a

			Cramer Class III and AChE Dimethoate (insecticide) 1.5 or 0.3 ug/kg bw d- 150 or 30 ug/kg bw d- 50 ug/kg bw d- 0.2 ug/kg bw d-[18] 86 ug/kg bw d-[19] 750 or 150 X Not a NOEL 250 X Not a NOEL! [1] Assuming a 60 kg person to distribute the TTC per Kg of body weight, to facilitate comparison across species. [2] We are conservative in falsifying TTCs based on the 5th percentile NOEL (we assume 100X UF), not a median NOEL. Anyway, the next column contains a chemical-specific claimed NOEL. [3] EFSA's draft opinion lists the 10% most-toxic NOELs for Cramer Classes I & III . Note the roughly 100-fold difference of a chemical's TTC and its NOEL, reflecting the UFs used. [4] As aquatic toxicity studies are common, sometimes the lowest dose falsifying a claimed NOEL is in "ug/L" units. But these are roughly comparable to "ug/kg of b.w." units because one L of water has a mass of 1 kg, and a density similar to body tissue (and most aquatic organisms continually run water through their bodies). 5] Ohtani H, Ichikawa Y, Iwamoto E, Miura I. Environ Res. 2001 Dec;87(3):175-80. Effects of styrene monomer and trimer on gonadal sex differentiation of genetic males of the frog Rana rugosa. [6] Mao H, Fang X, Floyd KM, Polcz JE, Zhang P, Liu B Brain Res. 2007 Dec;1186:267-74. Induction of microglial reactive oxygen species production by the organochlorinated pesticide dieldrin. [7] Robinson DE, Henry C, Mansingh A Environ Technol. 2002 Nov;23(11):1275-84. Toxicity, bioaccumulation & tissue partitioning of dieldrin by the shrimp, Macrobrachium faustinum de Sassure, in fresh & brackish waters of Jamaica. [8] 30 day reproductive toxity to fish. Lamai SL, Warner GF, Walker CH Eco
69	4. EFSA's consideration of the human exposure threshold values	R.I.S.K. Consultancy	FULL (In-vivo only) FALSIFICATIONS of "Safest" All-Purpose TTC, Cramer-III: Including Endocrine Disruptors (which EFSA proposes to use this TTC for). Some findings even falsify the lowest (safest) TTC of all, for Genotoxic Carcinogens Note: these falsifying doses are all LOAELs, so the true NOAELs would be lower yet. (administration route varies, but many are by feed/gavage). TTC Category Chemical TTC value19 TTC assumed NOEL20 Most Potent Dose Found Num. of Times Lower EDC / Cramer Class III Diethylstilbesterol (DES) 1.5 ug/kg bw d- 150 ug/kg bw d- 0.018 ug/kg bw d-53 8333 X Bisphenol-A

			0.025[U1] ug/kg bw d-[1] 6000 X HCB + 123-TCBenzene 0.1 ug/kg bw d-[2] 1500 X BDE-47 0.2 ug/kg bw d-[3] 750 X Ethinylestradiol (EE2) 0.2 ug/kg bw d-[4] 750 X TriButylTin 0.4 ug/kg bw d-[5] 375 X Dicamba 0.9 ug/kg bw d-[6] 167 X Atrazine 1 ug/kg bw d-[7] 150 X Bisphenol-A 2 ug/kg bw d-[8] 150 X Fenarimol (pyrimidine fungicide) 2 ug/kg bw d-[9] 75 X BDE-47 2 ug/kg bw d-[10] 75 X Deltamethrin 3 ug/kg bw d-[11] 50 X Di-n-butyl phthalate 10 ug/kg bw d-[12] 15 X Perchlorate 10 ug/kg bw d-[13] 15 X PFOA 10 ug/kg bw d-[14] 15 X

			Octylphenol 10 ug/kg bw d-[15] 15 X Methoxychlor 10 ug/kg bw d-[16] 15 X o,p'-DDT 18 ug/kg bw d-[17] 8 X Methoxychlor (2, at same dose) 20 ug/kg bw d-[18] 8 X Toxaphene 50 ug/kg bw d-[19] 3 X BDE-99 60 ug/kg bw d-[20] 21/2X [1] Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice..Endocrinology. 2005 Sep;146(9):4138-47. [2] Z. Valkusz, i, M. Gálfi et al., Further analysis of behavioral and endocrine consequences of chronic exposure of male Wistar rats to subtoxic doses of endocrine disruptor chlorobenzenes, Physiology & Behavior 103 (2011) 421–430. [3] Nadia Abdelouahab, Alexander Suvorov, Jean-Charles Pasquier, Marie-France Langlois, Jean-Paul Praud, Larissa Takser, Thyroid Disruption by Low-Dose BDE-47 in Prenatally Exposed Lambs, Neonatology 2009;96:120–124 [4] Mélanie Vosges, Jean-Claude Braguer and Yves Combarnous, 2008. Long-term exposure of male rats to low-dose ethinylestradiol (EE2) in drinking water: Effects on ponderal growth and on litter size of their progeny, Reproductive Toxicology:25:2:161-8. [5] Meador JP, Sommers FC, Cooper KA, Yanagida G. Environ Res. 2011 Jan;111(1):50-6. Tributyltin and the obesogen metabolic syndrome in a salmonid. [6] M Fernanda Cavieres, J Jaeger, & W Porter Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size. Environ Health Perspect. 2002 Nov; 110(11): 1081–5. [7] V. Belloni, E. Alleva, F. Dessì-Fulgheri, M. Zaccaroni and D. Santucci, Effects of low doses of atrazine on the neurobehavioural development of mice, Ethology Ecology & Evolution 19: 309-22, 2007. [8] R Melnick, G Lucier, et al. 2002 Apr;, Summary of the National Toxicology Program's Report of the Endocrine Disruptors Low-Dose Peer Review, Environ Health Perspect:110(4):427-31 [9] Mira Park, Jiyou Han, Jeong-Jae Ko, Woo-Sik Lee, Tae Ki Yoon, Kangseok Lee, Jeehyeon Bae, Maternal exposure to fenarimol promotes reproductive performance in mouse offspring, Toxicology Letters 205 (2011) 241– 249 [10] Alexander Suvorov and Larissa Takser, Delayed response in the rat frontal lobe transcriptome to perinatal exposure to the flame retardant BDE-47, J. of Appl. Toxicology, 11 March 2011 onli

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71	4. EFSA's consideration of the human exposure threshold values	R.I.S.K. Consultancy	[Cont"d] [9] Alyea RA, Watson CS Environ Health Perspect. 2009 May;117(5):778-83. Differential regulation of dopamine transporter function and location by low concentrations of environmental estrogens and 17beta-estradiol. (note: 1 picoMole *381 pg) of dieldrin in vitro caused neuronal cell biochemistry to go haywire after about 1 minute, due to sensitive cell membrane signaling). [10] Edwards R, Millburn P, Hutson DH. Toxicol Appl Pharmacol.1986Jul;84(3):512-22. Comparative toxicity of cis-cypermethrin in rainbow trout, frog, mouse, and quail. (note: the delivered dose causing these in vivo brain concentrations would certainly be below this chemical's falsified NOELs, as this concentration was highly toxic in vivo--severe convulsions, etc.). [11] Kim Y, Jung J, Oh S, Choi K J Environ Sci Health B. 2008 Jan;43(1):56-64. Aquatic toxicity of cartap & cypermethrin to different life stages of Daphnia magna & Oryzias latipes. (note: other endpoint & species toxicities were also potent, though none as potent as this). [12] Anton PM, Theodorou V, Bertrand V, Eutamene H, Aussenac T, Feyt N, Fioramonti J, Bueno L Dig Dis Sci. 2000 Sep;45(9):1842-9.Chronic ingestion of a potential food contaminant induces gastrointestinal inflammation in rats: role of nitric oxide and mast cells. [13] Bhuyan DK, Bhuyan KCFree Radic Res Commun. 1991;12-13 Pt 2:621-7. Oxy radicals in the eye tissues of rabbits after diquat in vivo. (note: single application of as little as 12 to 25 ug (0.012 - 0.025 mg) and no more than 61 ug of diquat . A cornea weighs far less than a kg, but as this single dose of diquat caused permanent optical damage, we will assume these two factors roughly cancel each other, allowing us to roughly compare to the claimed NOEL with its units). [14] Sánchez M, Ferrando MD, Sancho E, Andreu E Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1999 Nov;124(3):247-52. Assessment of the toxicity of a pesticide with a two-generation reproduction test using Daphnia magna. (note: more toxic in later genertns!) [15] Sánchez M, Ferrando MD, Sancho E, Andreu Ecotoxicol Environ Saf. 2000 May;46(1):87-94. Physiological perturbations in several generations of Daphnia magna straus exposed to diazinon.

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72	4. EFSA's consideration of the human exposure threshold values	R.I.S.K. Consultancy	The OECD/GLP protocol chronic tox. tests that many of the NOELs the TTCs rely on have the following deep flaws: - Are performed by the party with a massive interest in the agent being declared safe enough to market; - Test only a small & irrelevant part of possible doses (doses near poisoning, due to expense of larger number of animals to detect smaller disease increases); - Study only a few endpoints; and primitively so (the light microscope, mostly); - Often do not test the vulnerability of developmental exposure; - Are unable to, and do not even try to test what happens after our continual & ever-shifting exposure to innumerable mixture of toxic chemicals in our environment; - Always kill the animals at about the human equivalent of 60 years old—ensuring that most of any induced disease has no chance to develop (unbelievable!). Thus a TTC 's underlying data is almost always unreliable; it never predicts a safe dose. Next I will provide specific falsifications of various claimed safe TTCs.
73	4. EFSA's consideration of the human exposure threshold values	R.I.S.K. Consultancy	If we don't limit indications of risk to live mammals, there are thousands of toxicity results at far, far lower doses. We chose not to summarize such results (aquatic organism, in-vitro, and other study types)--except in the previous table, where we falsify the very studies of chemicals the TTCs are based on. From these ultra-potent toxic doses it is evident that almost any chemical might be toxic very low dose indeed. Without explaining why, it is a fact that such very low dose risks are almost un-testable in expensive chronic mammalian toxicity tests, raising the need for precaution. We do acknowledge that potent in vitro toxicities may be compensated by defensive mechanisms in vivo, but the toxic doses of aquatic toxicity tests in vivo are very disturbing. Thus there are many more published falsifications of the TTCs, that we did not find or haven't the space to present—perhaps again as many in vivo studies (directly comparable to the regulatory NOELs) as the number we present here, and hundreds more in vitro and aquatic toxicity findings [e.g. there are at least 40+ aquatic toxicity tests of OP (not even carbamate) insecticides falsifying the AChE TTC]. And so many aspects of toxicity remain unstudied, especially the effects of full-lifespan realistic doses, that it is only rational to assume many more falsifications of the TTC, even by vivo tests. So long as a chemical has been studied in academia, experience shows there will always be high quality published studies that give some indication—at least in vitro--that the claimed NOELs may be false…enough reason to do better in vivo tests before approval. And often enough in vivo findings directly falsify the self-interested claims of safety by a chemical's producer; as I also show here. Given all this, there is every reason to believe that low-dose toxicities of agents we did not evolve with will continue to be uncovered. In sum, a more precautionary RA system is needed. Note: Finding a NOEL is the task job of RA (scientists), while setting a safe dose such as a TTC (adding the UF) is the job of risk managers (agencies). So I had to "back-out" each TTC's UF in order to compare it to the NOELs from our literature search (our table's "TTC's assumed

			NOEL", which we assume to be the typical of 100-fold UF). This assumption is conservative, because TTCs are based on the 5th percentile most toxic chemical, and we had to falsify that. Note 2: These tables add a handful of falsifications of the TTC's NOELs which PAN-E had not found by the time that we had to send PAN-E's 30 August 2011 TTC letter to European Commissioner for Health and Consumer Policy, Mr. Dalli
74	4.1. TTC values for potential (genotoxic) carcinogens	none	The proposal to use the TTC approach for genotoxic carinogens represents a move away from the long-established position in the EU of not setting limits for genotoxic carcinogens as safe levels of exposure cannot be clearly identified. There is uncertainty about the shape of the dose response curve at the low levels to which consumers may be exposed. The assumption (stated at line 721) that a linear relationship exists between exposure and response is not supported by any evidence. The linear extrapolation models used by the USA and proposed here have not been routinely used in the EU because of the uncertainty about low level dose response. It is correctly stated (lines 744-745) that linear extrapolation may lead to underestimation of true incidence of cancer, but it is also true that (depending on the shape of the response curve) that it may lead to an underestimation. On lines 755 to 774 it is demonstrated that there are genotoxic substances that could still present a carcinogenic risk at exposures of less than the proposed TTC of 0.15 microgram/person/day. The proposal to exclude known groups of particularly potent genotoxic carcinogens from the TTC does not seem to go far enough to ensure safety. No account is taken of untested substances that also might be particularly potent. The whole point of using the TTC approach is to give a way of estimating the safety of substances for which toxicological data is sparse, so having a TTC for genotoxic substances that does not cover all such substances seems to be pointless. The argument put forward by the Scientific Committee that it is unlikely that an untested substance would have a virtually safe dose of less than 0.15 microgram/person/day is not supported by the presentation of any evidence other than a reliance on the assumption of a linear dose-response relationship at low doses. The claim that "such an outcome would have a very low probability" appears to be unjustified. Whereas the TTC approach might be a useful tool for evaluation of incidents involving exposure to genotoxic contaminants, it is not appropriate for use in the authorisation process for substances intentionally used in or on foods and food-producing species (eg. food additives, animal feeds, pesticides, veterinary drugs, etc.). The such products there it is better to use the risk management tool of not authorising the use of genotoxic substances.
75	4.1. TTC values for potential (genotoxic) carcinogens	ANSES	Genotoxic compounds: the TTC value of 0.15 µg/person/day (or 0.0025 µg/kg bw/day) seems in conflict with the concept of Margin of Exposure, as developed by Efsa in its Opinion adopted in 2005 related to "A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic". Further consideration on this aspect should be welcomed to clarify the most appropriate concept to be used.
76	4.1. TTC values for potential (genotoxic) carcinogens	Confederazione Nazionale Coldiretti	line 776-786 Please clarify sources and public available documents for scrutiny. Please, refer to public and retrievable documents or papers, which underwent peer review. Furthermore, the paragraph describes in abstract terms that there would be "very low probability" of occurrence of substances with VSF lower than 0.15 nanograms/person per day, but no direct estimate of the probability is showed. The lack of comparable literature sources and the use of not strictly scientific framing could be ameliorated, also to give input to policy maker about the desirable levels of safety that the TTC method allows to reach.

77	4.1. TTC values for potential (genotoxic) carcinogens	UK Food Standards Agency	Lines 745-753 This text indicates that thresholds are expected even for genotoxic carcinogens, due to homeostatic and cytoprotective effects, and the abundance of cellular targets. Yet the conclusions given are not apparent from the cited 2005 SC Opinion. The text on homeostatic and cytoprotective effects, and the abundance of cellular targets, related to toxic effects other than genotoxicity (see the top paragraph of p7 of the 2005 Opinion). It was recognised later in the Opinion that threshold-based mechanisms are conceivable for genotoxic agents which do not react with DNA. Lines 791-796 The term “without appreciable risk” is used in the definition of the ADI, TDI etc. for chemicals which considered to exhibit thresholds, and the reference to there not being any appreciable cancer risk could be taken to imply a threshold for genotoxic carcinogens. Should this instead refer to “minimal” or “negligible” risk? Alternatively, the wording in Figure 2, page 47, appears more appropriate: “low probability of safety concern”. Should it be stated, consistent with the 2005 SC Opinion, that substances which are suspected of being genotoxic should not be deliberately added to food or used in the food chain if they leave residues? Lines 798-805 It is concluded that the TTC of 0.15 micrograms/day is sufficient to protect against heritable effects from genotoxic substances. However, this is based on limited data for 6 substances. Is this consistent with the conclusion that the Cramer class II TTC, which was based on data for 28 substances, is not sufficiently supported?
78	4.1. TTC values for potential (genotoxic) carcinogens	INRAN, Rome	Line 751 There is no scientific consensus at international level on this statement and this should be mentioned in the opinion. For some carcinogenic genotoxic substances the mechanism is not thresholded. Line 755-765 For transparency, the number of substances present in the CPDP which have a VSD below 0.15 µg/person per day (after exclusion of the groups of high potency carcinogenic substances) should be reported. Line 767 to 774 The conclusion of this paragraph should be that such strengthening of the scientific basis should be performed before the 0.15 µg/person per day threshold is used in EFSA Panels. Line 776 to 789 I would suggest this paragraph to be deleted because the use that has been done of statistics/probabilities by Munro (1990) and Fung et al (1995) is highly questionable and the presentation of their results in the opinion could be misleading for the reader. In order to quantify risk in the population we are not interested in the probability of a chemical substance picked up among the universe of chemical substances to be carcinogen. We are interested in the proportion of the population that is exposed through their diet to those substances that are indeed carcinogens. Consumers are exposed to a large number of substances, some of which have a VSD less than 0.15 µg/person per day. We do not know how many of them are in such situation and these substances can not be identified through chemical grouping. The carcinogen risk related to their presence can therefore not be quantified. However, it is clear that the more this 0.15 µg/person per day threshold is used for risk assessment of substances leading to the conclusion “no safety concern” (with no risk management action taken to reduce unintentional exposure and /or with authorization for new intentional use), the more consumers will be exposed to these substances.

			Line 791 to 796 It would be important to state that a low cancer risk (less than one in a million lifetime risk of cancer) related to each single substance, when multiplied by the overall EU population and when summed for all substances that could be assessed through the TTC approach in the future may lead to a number of cancers cases that risk managers might wish to take into consideration.
79	4.1. TTC values for potential (genotoxic) carcinogens	BASF SE	Page 18, line 707 ff, and Figure 2 page 47, line 2054 According to the decision tree proposed in the document a genotoxic alert would automatically trigger the TTC value of 0.15 µg/person/day, or 0.0025 µg/kg body weight per day (introduction and further elaborated in 4.1). If the exposure is greater the generic scheme for the application of the TTC approach (conclusion part, page 47) implicates the usage of a non-TTC approach (toxicological data, read across etc.). See figure 2, box entitled: Substance requires non-TTC approach (toxicity data, read-across etc.). It would be helpful to add to this document a proposed genotoxicity testing scheme, specifically addressing the origin of the structural genotoxic alert (which is most often based on an prediction of bacterial mutagenicity in Salmonella typhimurium, tested in an Ames assay). Based on this, a negative result in an Ames assay might consequently be used to prove the absence of a genotoxic potential of the test substance. Furthermore, a guidance on the requirement and applicability of read across in this context might be useful: Would a read-across based on structure similarities be regarded as sufficient, or would additional information from metabolism of the respective read across substances be required? A clarification of this question would be very helpful, possibly based on the presentation of some examples.
80	4.2. TTC values for non-cancer endpoints	BASF SE	page 21 table 3, line 857 In section 4.2 the TTC values for non-cancer endpoints are discussed in more detail. When evaluating Table 3 on page 21, the reported toxicological endpoints for the NOELs for the 613 substances in the database of Munro et al., 1996 are listed. According to this table, for four substances NOELs were based on endocrine. As endocrine is to our point of view no endpoint, but a mode of action, the question arises, whether the mentioning of this specific category adds anything to this assessment as it is most likely already considered under the endpoints reproductive, developmental, or other target organ toxicity. It is noted, that further down in the document it is noted, that the TTC concept is considered to be applicable also for substances with an endocrine mode of action.
81	4.2. TTC values for non-cancer endpoints	INRAN, Rome	Line 846 Could the SC state if it agrees with the use of NOEL/3 for sub-chronic studies as a proxy for NOEL of chronic studies and which is the argumentation to support this use. Would this rule be applicable more generally allowing to substitute chronic studies with sub-chronic studies? It would be important to state in the opinion for how many of the 613 substances the NOEL was established in this way. It is not clear from lines 848-849 if the database used to assess the 5th percentile contains adjusted NOEL or non adjusted NOEL. This need to be clear. Line 971 to 974 Could the SC state if it agrees with the adjustment of NOAELs to obtain estimated chronic NOAEL values by using a scaling factor of 6 for the results of the 28-day studies and a scaling factor of 2 for the results of the 90-day studies and which is the argumentation to support this use? Line 983 to 993 It should be specified if the NOEL are from chronic studies.

82	4.2. TTC values for non-cancer endpoints	Confederazione Nazionale Coldiretti	l 806-809 We wonder if the 613 substances used in Munro et al (1996) cover the entire chemical spectrum., and what assessments have been made to check against that. SC could also consider the evolution of substances released on the market in last 15 years and derive adequate inferecens.
83	4.2. TTC values for non-cancer endpoints	Confederazione Nazionale Coldiretti	ll 806-809 We wonder if the 613 substances used in Munro et al (1996) cover the entire chemical spectrum, and what assessments have been made to check against that. SC could also consider the evolution of substances released on the market in last 15 years and derive adequate inferecens.
84	4.2.3. Assessment of original papers and reports on substances in the lowest 10th percentile…	Confederazione Nazionale Coldiretti	4.2.3.1 ll 883-887 Coldiretti shares some doubts related to the truncation at the 5% of the tail of distribution with regard to substances considered to establish the NOEL.
85	4.2.3. Assessment of original papers and reports on substances in the lowest 10th percentile…	Confederazione Nazionale Coldiretti	4.2.3.1 line 899 EFSA's SC refers to grey literature or abstract. Since the TTC is a recent approach it should be based on rigorous and public literature subject to peer review as for ordinary academic papers. Please, refer to public and retrievable documents or papers, which underwent peer review. The value of peer reviewing is not only of scientific relevance, but attains to more general democratic scrutiny of the science by its wide community and serve the scope of acting independently and mostly, of being perceived independently (both of features are goals declared by EFSA in its Mission).
86	4.2.3. Assessment of original papers and reports on substances in the lowest 10th percentile..	Confederazione Nazionale Coldiretti	4.2.3.3 991 "It should be noted"

87	4.2.3. Assessment of original papers and reports on substances in the lowest 10th percentile…	Confederazione Nazionale Coldiretti	4.2.3.3 ll 970 Coldiretti wonders if EFSA's SC deems sufficient that on the absolute number of 861 studies reassessed from 1982 to 2008 only 85 studies provide 90 days trials on rodents. Consequently, Coldiretti wonders if this re-assessment of Munro DB is informative enough and allows for a fair comparison and validation.
88	4.3. Adequacy of TTC value in protecting against specific endpoints	R.I.S.K. Consultancy	As to EFSA's draft recommendation on allowing use of TTC for endocrine disrupting chemicals & developmental/reprotoxicity: EFSA appears to us to want to reduce one of the most critical of toxicity tests: reproductive & developmental, which often involves endocrine disrupting compounds (EDC). There has been a massive shift by academics to study the effects of developmental-age exposures, generating thousands of very low dose toxicity results; and EDCs especially have biologic reasons why they are often more toxic at low than at higher doses—e.g. receptor production shuts down when there are too many hormones for efficient signaling. /1/ Thus EDCs destroy the very paradigm of RA, "the dose makes the poison'! (dozens of papers already find low to be more toxic than higher doses). The EFSA draft opinion claims the TTC for EDCs is based on the independent toxicity literature, yet none of their three citations bear that out. More important, we know of no such lo-dose EDC study has been used in any regulatory approval of a chemical to be sold, and neither could 250 international, very experienced career risk assessors name such a RA, at Global Risk Assessment Dialogue, Brussels 2011, when asked in plenary by us, name any non-industry study ever used in any RA for decades! Even the industry club ILSI favored testing EDC risks! /1/ F. Vom Saal et al. 2002? 'A Physiologically-Based Approach to the Study of bisphenol-A and Other Estrogenic Chemicals On the Size of Reproductive Organs, Daily Sperm Production & Behavior' Toxicol. & Indus. Health:1&2:239-260; and F. Vom Saal et al. 1995 Toxicol. Ltrs. 77:343-350; and F. Vom Saal et al. 1997 'Prostate Enlargement in Mice Due to Fetal Exposure to Low Doses of Estardiol or Diethylstilbesterol, & Opposite Effect at High Doses' Proceed Nat. Acad. Sciences 94:2056-61
89	4.3. Adequacy of TTC value in protecting against specific endpoints	R.I.S.K. Consultancy	EFSA's Scientific Committee Opinion claims , lines 440-3) that the TTC is needed to relieve the growing mandates for more toxicity testi. Even the ever-improving detection of chemicals in the environment is cited as a pressure to use a simple TTC…though this is not a new development: these chemicals have always been there! The EFSA draft opinion reveals, I believe, EFSA's drive to eliminate a lot of toxicity testing. They agree with previous evaluations on where TTC use is inappropriate: for metals, proteins, polymers, bio-accumulating chems. And they recommend some new exclusions: chemicals with inadequately characterized SARs, on which many TTCs depend (Cramer Class II seems to be unreliable); nano-sized materials; irritants to the G-I tract; ionizing elements; and micronutrients (selenium, sodium, etc.—except when it is an essential metal salted with another ion). For all these exclusions EFSA says there is either not enough toxicity data to establish a TTC; or so much toxicity data that a TTC is unneeded. However, EFSA's scientific committee radically abandons caution by advocating the TTC's use for: 1) vulnerable infants & children; 2) endocrine disrupting & reproductive/developmental tests 3) the most potent genotoxic carcinogens n.b.: the opinion only explicitely recommends the TTC for 2)

			These are areas where even industry cautiously agrees that toxicity risks are too great for a TTC (e.g. ILSI not wanting to not apply the TTC in these areas), due either to potency or vulnerability; or where actual toxicity tests are badly needed (and increasingly mandated); or where there is already toxicity data (but it is ignored for not being GLP-compliant). Never should a chemical with any suspicion of affecting these vulnerable targets not be tested for toxicity!
90	4.3.2. Anti-cholinesterase-related neurotoxicity endpoints	Federal Office of Public Health	The TTC for organophosphates should be considered similarly as an acute reference dose (ARfD). It should be emphasized that an exposure scenario with lifetime of exposure is not appropriate but more an acute or subacute exposure scenario (e.g. international estimated short-term intake, IESTI).
91	4.3.3. Reproductive and developmental toxicity	Confederazione Nazionale Coldiretti	ll 1125 Is this public literature? Or retrievable? EFSA should consider better data and literature under conditions of public scrutiny.
92	4.3.3. Reproductive and developmental toxicity	UK Food Standards Agency	Lines 1104-1110 Developmental toxic effects are generally considered relevant to acute exposure, except for effects which are considered secondary to maternal toxicity (Solecki et al. Fd. Chem. Toxicol. 43:1569-1593, 2005). Since acute exposures are usually higher than chronic exposures, in some cases considerably higher, the conclusion that the TTC values are protective for developmental effects implies that acute exposure should be compared to the TTC in order to ensure that developmental effects are protected against. Does this affect the conclusion on the use of the TTC for exposure periods which are less than chronic (lines 1582-1585)?
93	4.3.3. Reproductive and developmental toxicity	ILSI Europe aisbl	Lines 1079-1082 These sentences may not only refer to work done by Kroes et al. 2004 on teratogens, but also to the Munro 1999 (Food Chem. Tox. 37: 207) publication? The Kroes et al. 2004 analysis concluded that, based on the exclusion of high potency carcinogens early in the decision tree, any remaining teratogens would be addressed adequately by the threshold-based Cramer/Munro classes. Lines 1089-1110 The opinion presents the result of a comparison of the Cramer/Munro thresholds with NOELs of substances classified in the EU for reproductive or developmental hazards. Would it be possible to present or reference the data used, especially the source of the NOELs and how those were selected?
94	4.3.4. Substances with endocrine-modulating activity	INRAN, Rome	There is clearly no consensus in the scientific community on this issue. This paragraph should be implemented to better reflect the diverging views inside the scientific community by including reference to some significant peer reviewed papers on low dose effects. The document should underline that many knowledge gaps exist about endocrine-related effects on developmental programming, either in prenatal and post-natal phase and that NOEL for specific adverse effects might be much lower than those for conventional reproductive endpoints. Extensive research is on going on this topic. It is stated in lines 1166 to 1168 that “current knowledge supports the proposition that existing TTC values will also cover many endocrine mediated

			adverse effects, particularly those involving reproduction, development and thyroid function.". It might be more appropriate to say that it covers "some" of these effects. In any case covering "many " or "some" endocrine mediated adverse effects is clearly not sufficiently protective. I would therefore suggest that the conclusion is modified and that the use of TTC approach for these substances is not appropriate at the current state of knowledge.
95	4.3.4. Substances with endocrine-modulating activity	ANSES	Endocrine disruptors: We acknowledge that EDs are included in the document. For many EDs (ie bisphenol A, DEHP), the current health-based guidance values (TDI) are higher that the Class III TTC value (1.5 µg/kg bw/day) concurring with the fact that, as of today, ED are "covered" by the concept. Research on EDs continuously delivers scientific results and knowledge. Therefore, one can not rule out that, in the future, the TTC approach described here may need revisions when dealing with EDs. Consequently, we would suggest to include EDs in a specific box within the overall decision tree that the reader has to decide on the appropriate approach in the case of a potential ED.
96	4.3.4. Substances with endocrine-modulating activity	Danish Environmental Protection Agency	- P 27, line 1137-40: We agree that it is important to distinguish these kinds of interactions from adverse effects, however, until this area is more explored, it seems not scientifically sound to neglect these data. - P.27, line 1143-1150: Since adverse effects are a consequence of both potency and exposure, the fact that xenobiotics generally have less potent endocrine activity than endogenous hormones does not substantiate that xenobiotics cannot lead to adverse effects via food or the environment if the exposure is big enough. Further, the developing organism often does not have the compensatory feedback mechanisms necessary to compensate for impacts on the hormonal balance, and a lot of studies conducted during the last 10-15 years show that for endocrine disruptors critical windows of exposure (where minor imbalances in the endocrine system in a narrow period can lead to severe effects later in life) is of significant importance (besides potency and concentration). Further, focussing narrowly on substances acting in the same way as endogenous hormones is misleading. EDCs might also act through disruption of e.g. production, transport or breakdown of endogenous hormones. - P.28, line 1150-1154: The TG 407 is used to illustrate that compensatory feedback mechanisms are important. It is also used to show how difficult it is to use existing OECD Test Guidelines to identify ED effects since the effects are "too variable and insensitive". We agree that the OECD TG 407 is only able to identify certain types of strongly and moderately acting EDCs. But the reason, which should also be reflected, is that the use of mature animals with developed endocrine systems is a much less sensitive system than e.g. the developing fetus (se comment above). - P 28, line 1159-61: It is stated that only if the body is unable to regulate exposures within its limits of homeostasis is the threshold of adversity crossed. See comments above regarding feed-back mechanisms in developing organisms and critical windows of exposure. Further, we still don't know if there is a threshold for endocrine modulating (see also 4.4.1.6 regarding low-dose effects). - P.28, line 1161-1165: We strongly disagree to this statement. The hazard and risk assessment procedures in place in 1996, if based on the OECD Test Guidelines available at that time, did not really take endocrine disruptive effects into account. Even today, the guidelines developed in the last decade by the OECD EDTA to identify EDs are not routinely used in the hazard and risk assessment procedures e.g. under REACH (not part of standard information requirements). - P.28, line 1166-1177: Firstly, the Danish EPA agrees that some (few) EDs might be identified by use of some standard toxicology tests. However, many endpoints linked to ED modes of action are insufficiently covered by the existing standard test methods, including OECD TG 407, 408, 409,414, 415, 416, 452 and 453, even though some of them represent a higher tier of tests for detecting endocrine disrupting properties. The extended one-generation assay (EOGRTS, TG 443) recently adopted in the OECD provide substantial improvements regarding ED endpoints. Please consider to mention this study, which has a higher sensitivity for detection of EDC than the OECD TG 416.

			- In conclusion, we are of the opinion that this line of argumentation from EFSA SC is not up to the current state of science within this field today. We also do not find any persuading argument supporting the EFSA SC view expressed that the TTC concept can be used on EDCs. Finally, we note that the argumentation basically concludes based on older test guidelines and regulatory assessment schemes of the past and limits itself to primarily reproductive and developmental effects.
97	4.3.4. Substances with endocrine-modulating activity	RESEAU ENVIRONNEMENT SANTE	Line 1155-1165 There is no justification behind this statement of so-called absence of reproducibility of reported low-dose effects in experimental animal studies. For most studies, this is not true. The implications of low dose effects should be discussed in more detail in this EFSA opinion and be used for a better reflection of the limitations of the TTC approach. Line 1163-1165 This reference to the data of 1996 seems to us completely out of place considering the massive amount of research and knowledge on EDCs that has emerged since 1996. Line 1171 The two gen test is currently considered the critical test for hazard assessment of most endocrine parameters. This seems to be disputable as the 2 gen test has not been updated for years. In contrast, the extended 1gen test covers additional endocrine related endpoints. In general this chapter does not provide any convincing scientific evidence for the conclusion by EFSA that the TTC concept can be extended to EDCs. On the contrary, scientific evidence for non-monotonic dose response curves and new insights into the consequences of prenatal exposures are pretty much ignored. Therefore the basis for EFSA's arguments to extend the TTC approach to EDCs is flawed.
98	4.3.4. Substances with endocrine-modulating activity	WWF European Policy Office	Line 1155-1165 "This has been caused in part by the absence of reproducibility of reported low-dose effects in experimental animal studies." This sentence is not true in its generality. Only in some cases where low-dose effects have been reported, the findings have not been replicated (Melnick et al. Environ. Health Perspectives 110, 4, 2002). The implications of low dose effects should be discussed in more detail in this EFSA opinion and be used for a better reflection of the limitations of the TTC approach. Line 1163-1165 "However, such features have been assessed extensively and comprehensively in hazard and risk assessment procedures that were in place when the Munro database (1996) was compiled." It seems to be a false assumption that the data of 1996 already contain EDC specific features in a sufficient way. This would overlook the advances of scientific knowledge in this field over the last 15 years. Line 1171 "The two gen test is currently considered the critical test for hazard assessment of most endocrine parameters." This seems to be disputable as the 2 gen test has not been updated for years. In contrast, the extended 1gen test covers additional endocrine related endpoints. In general this chapter does not provide any convincing scientific evidence for the conclusion by EFSA that the TTC concept can be extended to EDCs. On the contrary, scientific evidence for non-monotonic dose response curves and new insights into the consequences of prenatal exposures are pretty

			much ignored. Therefore the basis for EFSA's arguments to extend the TTC approach to EDCs is flawed.
99	4.3.4. Substances with endocrine-modulating activity	UK Food Standards Agency	Lines 1174-1177 "…endocrine-mediated adverse effects are likely to be covered by the existing TTC values." While this may be true of many environmental chemicals, it may not be true of chemicals with high potency for endocrine activity, e.g. certain pharmaceuticals. For example, 17B-oestradiol has an ADI (JECFA) of 0.05 micrograms/kg bw/day, which is 30 times lower than the Cramer class III TTC. The glucocorticoid dexamethasone, which is used in veterinary medicine, has an ADI (EMA CVMP) of 0.015 micrograms/kg bw/day, which is 100 times lower than the Cramer class III TTC. Should some caveat be placed on the conclusion that the TTC is likely to cover endocrine-related effects?
100	4.3.4. Substances with endocrine-modulating activity	RIVM	Section 4.3.4 Pg 28 Ln 1172 This sentence may be considered as to advocate for OECD 416. It might be useful to include also reference to the newly adopted OECD 443, which gives virtually the same information, but which is less demanding from animal welfare point of view.
101	4.3.4. Substances with endocrine-modulating activity	RIVM	Section 4.3.4 Pg 28 Ln 1172 This sentence may be considered as to advocate for OECD 416. It might be useful to include also reference to the newly adopted OECD 443, which gives virtually the same information, but which is less demanding from animal welfare point of view.
102	4.3.4. Substances with endocrine-modulating activity	Confederazione Nazionale Coldiretti	line. 1139-1140 Interferences with the ordinary metabolic condition, as expressed by any kind of detectable effect, may be the signal for hazard of chronic adverse effects. Hence, the distinction between "adverse effects" and observed effects should not be overstressed, in particular when referring to endocrine modulating activity substances which are not well known yet and may have long term effects.
103	4.4. Substances not suitable for the TTC approach	DuPont	Would be useful to consider refinements to the software implementation of Cramer to be able to identify obvious "categories that should be excluded from the TTC approach"
104	4.4.1. Categories previously recommended for exclusion by others	Federal Office of Public Health	Line 1201, 1202: In the TTC concept described by Barlow (2005), dioxins had been excluded not only because of bioaccumulat but also because of its high potency for carcinogenicity. Line 1215ff: It is not becoming clear whether steroids are still excluded from the TTC concept. How should substances be assessed which have strong structural similarities to pharmacological acitve substances? Should the read-across approach be preferred in such cases?

105	4.4.1. Categories previously recommended for exclusion by others	ILSI IFBiC	Lines 1204-1209: The TTC model is a useful tool to assess the risks of exposure to chemicals with little available toxicity information that enter the food supply at low levels. The TTC model could also be used to assess the risks of dietary exposure to low levels of proteins introduced into GM food crops, for any proteins that do not share the profile of known food allergens or mammalian toxins. Proteins introduced into GM crops to date are present at low (10-1 to 102 ppm) levels in the grain/seed of GM crops (Hammond and Cockburn - The safety assessment of proteins introduced into crops developed through agricultural biotechnology, in Food Safety of Proteins in Agricultural Biotechnology, CRC Press, NY 2008). Processing of GM crops into food can result in further reduction in levels of a functionally active proteins (Hammond and Jez, Food Chem Toxicol 49(4):711-21, 2011). Dietary intake of functionally active introduced proteins is orders of magnitude below estimated safe thresholds of exposure based on very conservative assumptions used to calculate TTC levels. Given the very low dietary exposures and the weight of evidence available on the safety assessment of all introduced proteins, additional use of animals to test safety would generally provide little useful additional information and should not be routinely recommended. The weight of evidence alluded to includes bioinformatic screening to assess whether the introduced protein is structurally or functionally related to known allergens or mammalian toxins, and whether it is related to those that have a history of safe use in foods. Information on the mode of action is also developed, and its potential for digestion by proteases as well as stability to food processing conditions is also assessed. This weight of evidence has been articulated in various guidance documents from Codex, EFSA, ILSI etc. Thresholds for acute and chronic exposure to proteins were estimated based on adaption of TTC methods and applied to proteins (Hammond and Cockburn, 2008). While the data available for proteins is not as extensive as that for chemicals, more information is potentially available. It has been reported that approximately 800 unpublished toxicology tests on 180 enzymes were carried out that raised no issues of toxicological concern (Spok, Safety regulations of food enzymes, Food Technol. Biotechnol., 44(2), 197, 2006). Hammond and Cockburn (2008), using very conservative assumptions, calculated acute and chronic thresholds of 17.9 mg/kg/day bw (1074 mg/adult person/day) and 2.49 mg/kg bw/day (149 mg/adult person/day) respectively. These threshold levels are far above estimated exposures to functionally active introduced proteins (Cry1Ab, CP4 EPSPS) that are left in food after processing of seed/grain from biotech crops. These levels were estimated to be 0.000045 and 0.000008 mg/kg bw/day (Hammond and Jez, 2011). If the intake of functionally active introduced proteins is in the µg to sub-µg /kg bw range, these exposures are far below the lowest threshold doses considered safe for the majority of chemicals. Lines 1207-1209: state that common protein components of food would be classified as class I and II (1800 and 540 µg/person/day safe thresholds respectively). The Dietary Reference Intake Committee of the Institutes Food and Nutrition Board has recommended a protein intake for a male adult of 56 grams/day. Proteins commonly consumed in food would exceed this safe threshold such as those in bovine milk. One cup of milk contains ~ 2.4 grams or 2400,000 µg of as1 casein, similar amounts of ß-casein, 768,000 µg of lactoglobulin etc. Consumption of one cup of bovine milk would thus result in the intake of common food proteins far in excess of the Cramer Class I and II thresholds.

106	4.4.1. Categories previously recommended for exclusion by others	none	In lines 1216 to 1217, reference is made to an estimated one-in-a-million lifetime risk of cancer. Although such quantitative risk estimates are routinely used in the USA, these estimates are not usually used in the EU because of uncertainties about the methodology used to give such quantitative estimates. Nevertheless, in this instance, it is important to give this information about high potency carcinogens that may still present an unacceptable cancer risk at doses less than the proposed TTC for genotoxic carcinogens. Can we be sure that it is only aflatoxin-like, azoxy- or N-nitroso-compounds are the only classes of chemicals that will present an unacceptable risk of cancer at doses less than the proposed TTC?
107	4.4.2. EFSA considerations of categories previously recommended for.	RIVM	Section 4.4.2 intro Pg 29, line 1222. the wording “substances with endocrine activity” would also cover hormones although these were already excluded by Cramer. In addition the reasoning on lines 1155 to 1161 would equally well apply to hormones. The problem with hormones is most prominently present when these are given parenterally. Normally hormones will be quite efficiently be inactivated on first-pass and it is questionable whether natural hormones would indeed be much more (or unpredictably more) toxic than predicted by TTC values when taken orally. However, since TTC is most suitable to evaluate exposure to substances for which no or limited data are available, one might question whether exposure to hormones should be evaluated based on substance-specific data rather than on the basis of TTC. In addition, hormones is a term too general, in the sense that also for plants and insects hormones are known, and these might well be evaluated using TTC. Ln 1225-1226: This sentence seems to be connected to lines 1193-1194. It is questionable whether free metal ions (from organic bases or acids) should be submitted to the TTC approach. Free metal ions from such sources are no different from free metal ions from inorganic salts, for which exclusion is proposed in lines 1185-2287 and endorsed in line 1221. For some of such metals (e.g. Na, K, Mg, Ca) one might not be that concerned (usually there are already (group) ADI / TDIs even “unspecified” for these), but for other metals or metalloids (Fe, Cr, Co, Zn, Cu, Se) toxicity cannot be excluded (e.g. the SCF/NDA opinions on essential nutrients) and usually there are sufficient data to evaluate the safety of such metals at low levels of exposure. Therefore, it is recommended to exclude all metals from the TTC approach. For organic salts simply dissociation can be assumed (or should be demonstrated) after which the organic part can be evaluated using TTC and the metal ion can be evaluated based on element-specific data. For non-essential metals the data in the TTC database are to scanty to state that these are supported and even if ample data in one or two metals were available and included in the TTC database, it would make no sense to use this information to defend use of TTC for other non-essential (heavy) metals. Section 4.4.2.1 Pg 30, line 1255-1261 For a better understanding, it would be helpful if an adequate definition of bioaccumulation for mammals (including humans) is given in the document. For organisms in the environment (in particular for aquatic organisms), there is a common understanding of the meaning of bioaccumulation. According to REACH Annex XIII, a substance is considered bioaccumulative (B) if its BCF value is greater than 2000 l/kg and very bioaccumulative (vB) if it is greater than 5000 l/kg (or as screening criterion: Kow > 4.5). For mammals, the concept of evaluation of bioaccumulative potential based on Kow is rather simple. The criteria for bioaccumulation are not very specific and leave much room for interpretation. Kow in itself is a bad predictor of accumulating potential. The prediction of bioaccumulation potential would improve if the requirements poor metabolism were connected to this criterium (e.g. high Kow + steric hindrance and / or high KoW + chemical stability). For the moment the text seems to be an either/or-construction. It is noted though that the TTC concept has an inherent uncertainty. One might argue that simply using the three bullets separately would not violate this and would tend to be conservative. It is further noted that in REACH-Guidance Document R11 further screening criteria are provided regarding molecular size and weight,

			Kow and octanol solubility. Section 4.4.2.2 Ln 1273 Reference to 4.8 should be to 4.9
108	4.4.2. EFSA considerations of categories previously recommended for…	RIVM	Section 4.4.2.3 Pg 30, line 1274 – 1278. This is a hazard-based assumption. It is very unlikely that substances will cause severe GI tract irritation, let alone corrosion, at or below the TTCs. Actually many substances in food would cause GI-tract irritation (e.g. acetic acid, lactic acid) especially when given as a bolus, but still they can be safely eaten in food. Not even NaF given in drinking water (which will generate HF in the stomach) has produced severe effects at dose levels even higher than 100 * the class III TTC for life-time although some inflammatory effects on the stomach were observed. In addition, in many gavage studies GI-tract irritation is a well known phenomenon which would either be included in the evaluation of the NOAEL / BMDL or alternatively would trigger a feeding study being carried out. Also if the effect is still observed in such a follow-up study ,it will not be dismissed, but included in the study outcome. Hence: GI-tract irritation must have been be covered in the database underlying TTCs and there does not seem to be a special reason to exclude anticipated (or known) irritating substances from the TTC approach, at least not when oral exposure is considered. The situation may be different for dermal or inhalation exposure and this is an argument not to apply the oral TTCs for route-to-route extrapolation purposes (as explained in lns 1640-1641). Section 4.4.2.6 Pg 31, line 1289 – 1292 This paragraph is contradictory to lines 1225 – 1226 (see also our comments for pg 29).
109	4.4.2. EFSA considerations of categories previously recommended for exclusion and recommendations....	WWF European Policy Office	Line 1256 The BCF approach as an indicator for bioaccumulating substances is too limited as it is not a good surrogate for biomagnification: The BCF quantifies chemical bioaccumulation from water but not from the diet. Other parameters such as the octanol-air partition coefficient as well as in-silico modelling and in-vitro assays are needed to cover the majority of substances with bioaccumulation potential (see Science-Based Guidance and Framework for the Evaluation and Identification of PBTs and POPs:, Summary of a SETAC Pellston Workshop, 2008, http://www.setac.org/sites/default/files/ExecutiveSummary.pdf).
110	4.4.2. EFSA considerations of categories…	INRAN, Rome	It is suggested to add substances with endocrine-modulating activity to the list of substances to be excluded from the TTC approach.
111	4.4.2. EFSA considerations of categories previously…	Confederazione Nazionale Coldiretti	line 1219-1223 It is not clear in the formulation if the SC underpins exclusion of endocrine active substances from TTC approach.

112	4.4.2. EFSA considerations of categories previously ……	Confederazione Nazionale Coldiretti	line 1264-1273 As a rule of thumb-, it should be clearly evaluated if the substances toxicity curve distribution tested by Munro, Kroes et al. have been assessed versus the curve of distribution of the 50.000 and more substances released each year on the market (flavorings and others),at least for last 10 years- in order to see if data are comparable, even in the tails. If not, they should. We found documents (EPA 2000, EPA 2005) in which the chemical structure (the major basis for TTC) lacks of absolute predictability with regard to outcome not related to genotoxicity, and could be used for "provide valuable initial information"only. If this holds true, the SC should consider it. Some authors doubt also on the predictability of genotoxic effects as well (Snydera 2005).
113	4.4.2. EFSA considerations of categories previously …	UK Food Standards Agency	Should pharmacologically active substances be excluded from the TTC? There has been a view in the veterinary medicines field that further work would be required to assess whether the TTC would be adequate for pharmacologically active substances. For example, the ADI for the glucocorticoid dexamethasone (EMA CVMP) is 0.015 micrograms/kg bw/day, which is 100 times lower than the Cramer class III TTC. This may have implications for use of the TTC by FEEDAP.
114	4.4.2. EFSA considerations of categories previously ..	RESEAU ENVIRONNEMENT SANTE	Line 1256 The BCF approach as an indicator for bioaccumulating substances is too limited as it is not a good surrogate for biomagnification: The BCF quantifies chemical bioaccumulation from water but not from the diet. Other parameters such as the octanol-air partition coefficient as well as in-silico modelling and in-vitro assays are needed to cover the majority of substances with bioaccumulation potential (see Science-Based Guidance and Framework for the Evaluation and Identification of PBTs and POPs:, Summary of a SETAC Pellston Workshop, 2008, http://www.setac.org/sites/default/files/ExecutiveSummary.pdf).
115	4.4.2. EFSA considerations of categories previously recommended for exclusion and recommendations for additional exclusions	ILSI Europe aisbl	1276-1278 The aspect of local effects on the GI tract may need more explanation or specific consideration. Does the SC have a specific substance class in mind for which examples are available, where a substance at TTC-level exposure levels, i.e. at a maximum of 1800 µg/day or 1.8 µl/day would cause GI tract irritation? Most cases in which the TTC concept might be applied deal with substances present at trace levels, at which even strong acids or bases would not raise a concern for irritation, so that this general exclusion seems disproportionate. Would it be more appropriate to require an assessment of the probability of local effects at the maximum concentrations occurring in the respective exposure case? Another possible analysis would be to examine the studies of the Munro database with regard to the occurrence of local effects on the GI tract. Most probably, those would have been detected (via necropsy findings, pathology findings or organ weights) in the studies as well, even if they were not the most sensitive effect driving the NOEL, so that they may be covered by the Munro database. Munro 1996 does not seem to mention to have excluded GI tract effects /local effects from the database.
116	4.4.2. EFSA considerations of categories previously …	UK Food Standards Agency	Lines 1264-1273 It is unclear if the underpinning databases contain adequate numbers of toxins, e.g. mycotoxins, biotoxins, plant toxins. These can be complex molecules (not proteins), and can be highly toxic.

117	4.4.2. EFSA considerations of categories previously…	Federal Office of Public Health	Line 1233-1261: It should be described more clearly how to proceed with substances that are predicted to have (bio)accumulation properties in the TTC concept. The terms "accumulation" and "bioaccumulation" should be clearly defined and be adequately used in the text.
118	4.5. Adaptation of the TTC values for infants and children	Confederazione Nazionale Coldiretti	line 1304, 1320 Please, refer to public and retrievable documents or papers, which underwent peer review. The value of peer reviewing is not only of scientific relevance, but attains to more general democratic scrutiny of the science by its wide community and serves the scope of being perceived acting independently (goal declared by EFSA in its Mission).
119	4.5. Adaptation of the TTC values for infants and children	INRAN, Rome	Line 1342-1343 It would be useful to find other references in addition to Renwick et al. (2000) to support the important statement that “reduced elimination and excretion is transient and that the toxicokinetic differences between young infants and children or adults is generally not more than 2 to 5 fold.” Is there a consensus in the scientific community on such ratios?
120	4.5. Adaptation of the TTC values for infants and children	RESEAU ENVIRONNEMENT SANTE	Given the presented evidence supporting the immature status of xenobiotic metabolising enzymes and elimination processes in this report, what is EFSA’s basis for concluding that TTC can be considered for infants and children instead of stating this as a clear limitation of the TTC approach?
121	4.5. Adaptation of the TTC values for infants and children	WWF European Policy Office	Given the presented evidence supporting the immature status of xenobiotic metabolising enzymes and elimination processes in this report, what is EFSA’s basis for concluding that TTC can be considered for infants and children instead of stating this as a clear limitation of the TTC approach?
122	4.5. Adaptation of the TTC values for infants and children	Confederazione Nazionale Coldiretti	ll 1320 Please, refer to public available and verifiable documents, as for ordinary references in any scientific work undergoing peer-review. Since the text is expressing critical aspects of elimination and clearance of xenobiotics in children, a more rigorous explanation should be provided, with further argumentation. Furthermore, no reference to age-classes are provided with reference to ILSI workshop, apart from the 12 weeks infants as the lower age considered.

123	4.5. Adaptation of the TTC values for infants and children	RIVM	Section 4.5 Pg 31-31, line 1320 – 1347 Although obviously differences in kinetics between various age groups do exist, it might be argued if generally applicable assumptions can be made. Some substances may be eliminated faster and other may be eliminated slower from infants / neonates than from adults. In addition, if it is believed that developmental and reproductive toxicity are adequately covered by the database, then implicitly also infant / neonatal exposure with insufficient or over-sufficient clearance should have been covered. It may be questioned if the approach suggested by the SC is not over-conservative. It should also be noted that TTC is supposed to be representative for life-time exposures. Interpreting an exposure estimate for children as being representative for life-time exposure is not correct since normally exposure of children rapidly declines with increasing age. This is another argument why comparing exposure estimates for children to TTCs has an inherent conservatism. In addition, like young children also young animals have usually higher exposures than adults (at least in the classical chronic feeding studies). Therefore, using TTCs for the evaluation of exposures in children may not be that inappropriate. Section 4.5 Pg31, line 1314-1318 (see also pg 12, ln 497) Although the origin of the default extrapolation factor of 100 has been explained, the rationale of using a factor 100 for the universe of chemicals in TTC context is not discussed in this document. At least a discussion is needed on why chemical or chemical group-specific factors cannot be applied. It is noted that section 4.5 does discuss the default assessment factors used for infants and children.
124	4.5. Adaptation of the TTC values for infants and children	R.I.S.K. Consultancy	EFSA's draft opinion lays down copious words on adjusting the TTCs for infants (TTCs are almost entirely based on adult toxicity data. Unbelievably, they fail to discuss the far greater vulnerabilities of infants to chemicals, which range from higher intake rates & increased exposure (crawling, hand-to-mouth) to undeveloped organs and systems, such as the immune systems…not to mention their vastly increased expression of DNA, a massive increase in nuanced biochemistry that is a correspondingly great vulnerability to toxics./1/ So even if adult toxicity data were perfect and weight adjustment for children, it is absurd to claim that are "adequate for infants"! /1/ Landrigan PJ. 1999. Risk assessment for children and other sensitive populations. Ann N Y Acad Sci.;895:1-9.
125	4.6. Expression of TTC values on a body weight basis	RIVM	Section 4.6 Pg 32, line 1350 - 1351 These lines seem contradictory to what is stated under lines 1343 – 1347.
126	4.6. Expression of TTC values on a body weight basis	ANSES	p 32, lines 1352-1354: The sentence dealing with the need for comparing exposure for different age groups with the TTC value converted in µg/kg bw/d needs clarifications. A corresponding table could be introduced or completed (table 8) with the corresponding age groups and default value taken from the EFSA scientific committee guidance document for body weight (infants until 12 months; children over 3 years and adults).

127	4.6. Expression of TTC values on a body weight basis	Fraunhofer ITEM, Hannover	The EFSA Scientific Committee "concluded that the TTC values should be converted to a µmol/kg body weight basis for comparison with exposure estimates for different age groups" (page 32). We would further propose to use general TTC thresholds on a molecular basis (µmol/kg bw/d) as it is also indicated on page 48 of the EFSA document. Molar doses represent the absolute concentrations of substances and thus allow a direct comparison of the amount of substances (independent of their molecular weight). Therefore, molar doses describe toxicological potencies more precisely than body doses in mg/kg bw/d if substances of different molecular weights are analyzed. Threshold values based on NOELs in mg/kg bw/d are, thus, directly dependent on the molecular weight of those substances, which determine the 5th percentile in the respective database. An accurate substance specific threshold can then be calculated using the specific molecular weight of the target chemical and the average body weight of the target group. New proposed refined TTC values in µmol/person/ d for the three Cramer classes were derived in the submitted paper of Tluczkiewicz et al., 2011. Tluczkiewicz, I., Buist, H. E., Martin, M. T., Mangelsdorf, I. and Escher, S.E., 2011. Improvement of the Cramer classification for oral exposure using the database TTC RepDose – a strategy description. Submitted to Regul. Toxicol. Pharmacol.
128	4.7. Genotoxicity prediction tools	none	Lines 1376 to 1378 state that it is outside the scope of the document to give guidance on which QSAR tool to use. Nevertheless, much of the argument for setting a TTC for genotoxic carcinogens is based on conclusions drawn from application of a linear dose extrapolation to low levels of exposure, thus making assumptions about low level dose-responses that are out of line with the position normally taken by the EU that it is inappropriate to use linear extrapolation to estimate low dose risk to consumers from data generated at high doses in animal studies.
129	4.7. Genotoxicity prediction tools	R.I.S.K. Consultancy	The special case of TTC for genotoxic carcinogens is both a theoretically and an empirically horrible use of the TTC. The EFSA draft opinion claims they, as most previous TTC evaluators, do not think use of the TTC is safe here …but then they say it is, and present data showing that this TTC of 0.15 ug d- (0.0025 ug/kg bw d- for a 60 kg adult), is conservative enough to use on these potent carcinogens. But for decades such DNA-damaging suspected carcinogens have been regulated stringently with a 'linear no-threshold' (LNT) model: assumed to increase cancer down until zero dose, because it was known by the 1950's that just one cancer-causing mutation in one regular (non germ-cell) cell might propagate until a cancerous tumor develops (in reality this cautions RA approach should have been applied to the many more diseases that genetic mutations cause, not to cancer alone; in addition cancer arises from various non-genotoxic pathways). EFSA and industry's ILSI seem to be collaborating in an effort to use the TTC's "assumed safe" dose to negate the conservative nature of genotoxin carcinogens./1/ This would demolish the conservative LNT, substituting a 'threshold' (i.e. "safe") dose model. This is the only motive we can conceive for both ILSI-EU and EFSA proposing to apply the TTC to this category of chemicals, when no other evaluator of the TTC thinks it is appropriate to abandon the conservative LNT model! /1/ Barlow S, Schlatter J. 2010 Mar 1 Risk assessment of carcinogens in food. Toxicol Appl Pharmacol;243(2):180-90

130	4.7. Genotoxicity prediction tools	Confederazione Nazionale Coldiretti	ll. 1360 and 1373 We think that the specific decision tree, if useful as a screening tool has some drawbacks. In particular, in the fastest and shortest loop, the question about the presence of genotoxic available data/alerts is misleading. The absence of genotoxic data may be either due to the lack of a genotoxic profile or simply being a false negative due to the lack of data available yet. Referring to a previous EFSA's opinion (2005 Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic), we argue that " In cases where limited data on genotoxicity are available, e.g. only in vitro test results, the overall weight of evidence for genotoxicity should to be evaluated on a case by case basis taking into account other relevant information (e.g. chemical reactivity, metabolic fate)".Furthermore, we are concerned about ll 1373 declarations and 1377 ("further work is needed in this area"). It is not clear, based on the evidence available and after EFSA's own statements in the document, if EFSA's SC deems sufficiently established and grounded the approach stemming from the decision tree. It seems there is some incongruence between assumptions and conclusions; between the lack of predictability in most of the cases and the practical use that can be done and which is recommended.
131	4.7. Genotoxicity prediction tools	Federal Office of Public Health	It would be useful to the reader to know how to proceed in case of a positive finding by a genotoxicity prediction tool. Which set of in vitro genotoxicity tests should be performed to rule out genotoxicity? It could be taken reference to the new EFSA Scientific opinion on genotoxity testing strategies applicable to food and feed safety assessment. See also line 514-516.
132	4.7. Genotoxicity prediction tools	DuPont	Line 1373 Relatively few models exist which purport to predict in vivo genotoxicity let alone accurately. The majority of models that do exist consider the prediction of Ames mutagenicity.
133	4.8. Metabolic prediction tools	ILSI Europe aisbl	Lines 1397-1413: It might be helpful to provide some additional perspective on the necessity of metabolic prediction tools for the identification of genotoxicity alerts. There is no doubt that the recent and future development of enhanced metabolic prediction tools will facilitate and improve the assessment of genotoxicity and non-cancer endpoints. However, already the genotoxicity alerts described by Ashby and Tennant include structures which require metabolic activation. Expert-rule based and other SAR systems consider metabolic activation and the Ames study results used as training sets for SAR systems originate from studies with and without metabolic activation. Therefore, while there is much room for improvement, it should be pointed out that metabolic activation was not ignored in the hitherto approaches.
134	4.8. Metabolic prediction tools	RIVM	Section 4.8 Pg 33, line 1387 – 1388 The JECFA statement includes implicitly a risk assessment for the anticipated metabolites an the wording actually promises much more than can be given. Even if metabolism data for structurally related substances are available, it remains tricky to predict the metabolism of a substance under evaluation, although of course general principles can be applied (e.g. conjugation of hydroxy groups, oxidation of alcohols, aldehydes and many more). However, predicting hazards of anticipated metabolites is as good as QSARs generally are, and indeed quantitative prediction of metabolism and metabolite patterns is virtually impossible. Any statement on potency of an anticipated metabolite is sheer guessing. It is therefore rather difficult to use this statement. Probably the best use of metabolism data is to support read-across in case the exposure to a substance under evaluation is above the TTC and no substance-specific data are available. If genotoxicity is sufficiently covered there is no use to differentiate between an A-side or a B-side.

135	4.8. Metabolic prediction tools	RIVM	Section 4.8 Pg 33, line 1387 – 1388 The JECFA statement includes implicitly a risk assessment for the anticipated metabolites an the wording actually promises much more than can be given. Even if metabolism data for structurally related substances are available, it remains tricky to predict the metabolism of a substance under evaluation, although of course general principles can be applied (e.g. conjugation of hydroxy groups, oxidation of alcohols, aldehydes and many more). However, predicting hazards of anticipated metabolites is as good as QSARs generally are, and indeed quantitative prediction of metabolism and metabolite patterns is virtually impossible. Any statement on potency of an anticipated metabolite is sheer guessing. It is therefore rather difficult to use this statement. Probably the best use of metabolism data is to support read-across in case the exposure to a substance under evaluation is above the TTC and no substance-specific data are available. If genotoxicity is sufficiently covered there is no use to differentiate between an A-side or a B-side.
136	4.9. Chemoinformatic analysis of TTC datasets	ILSI Europe aisbl	1461 It would be helpful to include a definition as to what is regarded in this context as a “high” molecular weight.
137	4.9. Chemoinformatic analysis of TTC datasets	ILSI Europe aisbl	1461 It would be helpful to include a definition as to what is regarded in this context as a “high” molecular weight. 1504-1505 Quote: “They confirm the protectiveness of the Cramer scheme for both non-cancer and cancer endpoints.” Comment: Is this sentence meant to refer to the TTC scheme in general? Or to non-cancer and non-genotoxic carcinogen endpoints? It is unclear how the Cramer classification scheme would provide assertion concerning genotoxic endpoints.
138	4.9. Chemoinformatic analysis of TTC datasets	Xiphora Biopharma Consulting	Line 1437-1464 It is considered misleading to refer to the carcinogenicity dataset employed for chemoinformatic analysis as the "CPDB dataset". The derived dataset contains only 609 structures whereas the current CPDB comprises 1547 chemicals (around 50% of which are noncarcinogens). Not only is the derived dataset highly selective, it has been processed into "minimum corrected" TD50 values. On reading the Bassan report it seems that the TD50 values supplied by FDA were adopted without any independent checking and so the claim that the data were "quality-checked" seems hard to substantiate. I have performed an independent check on two compounds: methyl methanesulfonate and o-toluenesulfonamide. The CPDB shows TD50 values of 31.8 (http://potency.berkeley.edu/chempages/METHYL%20METHANESULFONATE.html) and 3960 (http://potency.berkeley.edu/chempages/o-TOLUENESULFONAMIDE.html) mg/kg/day respectively. On the other hand, Bassan et al cite their TD50s as 0.178 (compound 319) and 0.375 (compound 531) mg/kg/day respectively. MMS is a key compound in the context of genotoxic impurities in pharmaceuticals and the CPDB TD50 of 31.8 mg/kg/day is universally accepted; there is no obvious reason for using a value of 0.178 mg/kg/day, and this seems to be a transcription error. o-Toluenesulfonamide is Ames-negative and seems highly unlikely to be a potent carcinogen. Therefore, the TD50 value of 0.375 mg/kg/day seems highly suspect.

			A further issue is the inclusion of compounds (previously identified that are tagged as "carcinogenic" purely as a result of using a point estimate of the lowest statistically significant TD50. A number of these "statistical" carcinogens are still present in the Bassan database, eg adipamide (15), 3-nitropropionic acid (374), parathion (448), phenol (461). It appears that some of the original statistical carcinogens have been excluded however, such as endosulfan, rotenone, azinphosmethyl and fenvalerate. Overall, it is considered that the Bassan CPDB dataset is slightly improved compared to that used by Cheeseman (and presumably Kroes) but still compromised. A rigourous cross checking procedure is required rather than relying on the dataset supplied by FDA. Moreover, the use of point estimate lowest statistically significant TD50 values is NOT considered justified since this conflicts with current weight-of-evidence paradigms in the evaluation of genotoxicity and carcinogenicity data, and the use of such values is not particularly transparent. In the broader field of toxicology the headline CPDB TD50 (geometric mean) values are employed, and it is believed that we should strive for consistency in matters relating to carcinogenic potential. A further issue relates to data quality and integrity. Many of the carcinogenicity bioassay reports used as source documents for the CPDB are several decades old and would be considered inadequate (sometimes grossly so) by current standards. For example, the data on MMS originate from a 2-page Science report published by Clapp et al in 1968. This issue is rarely acknowledged; the CPDB seems to act somewhat as a "data washing" mechanism. It is considered inappropriate on the one hand to base regulatory standards on grossly inadequate studies, yet on the other hand to be highly critical of other (submitted) studies that may have slight deficiencies. Comments disputing the claim that the derived CPDB dataset is representative of the wider world of chemical substances can be found in an earlier part of my response.
139	4.9. Chemoinformatic analysis of TTC datasets	DuPont	Line 1445 Some substantiation of the statement "representative of the world of chemicals". A further statement to justify the representativeness of the DssTox chemicals with the Munro datasets on the basis of a small subset is merited. This is no doubt discussed in much more detail in the main report but the abstracted section provided in this opinion paper is unconvincing.
140	4.9. Chemoinformatic analysis of TTC datasets	DuPont	Line 1464 Unsurprising since CPDB are more likely to contain chemicals that are presupposed to be carcinogenic and hence a high number of PAHs is to be expected. Perhaps a note to qualify that this is not unexpected would be helpful.
141	4.9. Chemoinformatic analysis of TTC datasets	WWF European Policy Office	Line 1448 It is unlikely that a subset of round 500 substances drawn randomly covers the large variety of chemical structures, the high diversity of toxicological endpoints and the differences in vulnerability of for different groups exposed. Explanations on route-specific metabolic factors, pre-systemic metabolism, extrapolation routes, toxicodynamic and toxicokinetic show the complexity of aspects involved in the toxicity of substances. Therefore a scientifically robust statement about a substance requires a comprehensive assessment. Line 1504/1505 “They confirm the protectiveness of the Cramer scheme for both non-cancer and cancer endpoints”.

			This sentence is not backed by the scientific evidence presented in this approach. Which underlying value assumptions were made to conclude protectiveness? This is an intransparent and problematic statement, given that 5% percentile is used instead of lowest NOEL (which even also may be associated with effect levels of up to 20%, as recently stated by the EC scientific committees in their draft opinion on mixture assessment http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scher_consultation_06_en.htm).
142	4.9. Chemoinformatic analysis of TTC datasets	none	The Ames test was the only genotoxicity test considered. What about other genotoxicity tests, including those covering clastogenicity and aneugenicity, which cannot be taken into account in bacterial assays such as the Ames test.
143	4.9. Chemoinformatic analysis of TTC datasets	DuPont	Line 1470 Unsurprising that QSAR relationships could not be derived for TD50 or NOEL - given the myriad of mechanisms, modes of action as evidenced by the different target organs affected. A note to comment on this would be helpful.
144	4.10. Exposure	ANSES	p35 line 1518. It is stated that most of efsa panels use in their assessment average chemical concentration values, measured or predicted to estimate chronic dietary exposure. Not sure that the word “most” is currently reflecting those practices. If it is the case, panels should be listed in parenthesis to be transparent. P36 lines 1542- 1544. It should be added that the total diet studies are the most refined method for defining dietary exposure to chemicals as they allow to remove the background noise for the surveyed population. At the opposite of probabilistic methods which are not so often used in risk assessment procedure for substances added or present in food.
145	4.9. Chemoinformatic analysis of TTC datasets	RIVM	Section 4.9 Pg 35, line 1503-1505 It may be necessary to discriminate here between carcinogens with and without genotoxic properties, since otherwise these lines are in conflict with lines 1500-1501 where lower TTCs for genotoxic substances are advocated. Alternatively, it may be better to refer to the Kroes, rather than the Cramer scheme, because Kroes actually took genotoxicity into account, while Cramer did not. In addition Cramer only discriminated between Classes. The actual TTCs came from Munro.
146	4.10. Exposure	Confederazione Nazionale Coldiretti	Par. 4.10.2 line 1580-1585 SC proposal is welcome since it tries to provides evaluation of toxic effects versus duration of the exposure. However, the example at 1584-1585 could be made more clear to derive empirical indications.

147	4.10.1. Dietary intake estimates for TTC	INRAN, Rome	All over the opinion it is suggested to use the terminology “dietary exposure” instead of “dietary intake”. Line 1525-1538 The sentence “Such methods are often criticised for being overly conservative, especially for consideration of lifetime exposure” does not represent adequately concerns expressed in the scientific community. In a number of cases such methods have been shown not to be conservative enough. Thus the model used in the Food Contact Materials area (1 kg of food or beverage packaged with material containing the substance of interest) is not conservative in relation to the consumption of beverages in plastic bottles in the total population and is not conservative in relation to consumption of packaged food per kg body weight of infants and young children. Such potential for underestimate of exposure in children was mentioned in the EFSA Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment adopted in 2006 (table A1, p. 32-33). A review of the Norwegian Scientific Committee for Food Safety suggests that consumption of packaged food in infants (0-12 months) is up to 10 times (156 g/kg bw) the standard assumption of 1 kg in a 60 kg subject (Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety: Evaluation of the EU exposure model for migration from food contact materials. Available at http://www.vkm.no/dav/2a7d1ce442.pdf.) The paragraph should be implemented accordingly. Line 1580 to 1585 It is suggested to delete the sentences “It is therefore recommended that the issue of less than chronic exposure should be addressed case-by-case. This could be done for example by considering the margin between the appropriate TTC value (without any adjustment for duration of exposure) and the estimated dietary intake.” In fact, mentioning case-by-case opens the door to any interpretation and possible application. Assessing a margin between TTC value and exposure would be of interest if there was a scientific basis to judge such margin, which is not the case.
148	4.10.2. Duration of exposure	ILSI Europe aisbl	1563 In this context, it would be useful to review and cite work done by EMEA: EMEA 20101, EMA/CHMP/SWP/431994/2007 Rev, 3, dated 23-September 2010; Questions and answers on the "Guideline on the limits of genotoxic impurities" http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002907.pdf and the related "Guideline on the limits of genotoxic impurities" http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf 1580-1585 It is important to clarify that the proposal of Felter et al. (2009) was in regard to less-than-lifetime exposure to carcinogens, but did not specifically address acute exposure. This said, a TTC of 1.5 ug/d would be regarded as being highly conservative for virtually all acute exposures. It is certainly prudent to recommend that the issue of less-than-chronic exposure be addressed on a case-by-case basis. A recent review article has been published in Critical Reviews in Toxicology that provides substantial background on this topic and is an important reference to include: Felter, S.P., R.B. Conolly, J.P. Bercu et al., 2011. A proposed framework for assessing risk from less-than-lifetime exposures to carcinogens. Crit. Rev. Toxicol., 41(6), 507-544.

149	4.11. Routes of exposure other than oral	INRAN, Rome	Line 1790 to 1808 The SC consideration of non dietary sources of exposure is highly welcome. Difficulties in establishing TTC values for dermal exposure should not prevent from considering that dietary exposure is not the unique source of exposure in the risk assessment. Therefore for substances for which inhalation exposure and dermal exposure are known to be significant, one could consider that the full TTC should not be covered by dietary exposure.
150	4.11. Routes of exposure other than oral	Confederazione Nazionale Coldiretti	Coldiretti is particularly interested also in workers' exposure and supports an approach considering also several routes of exposure to substances. Assessing environmental exposure other than from food, long-term exposure should be considered.
151	4.11.1. Existing databases for TTC - non-cancer endpoints	Confederazione Nazionale Coldiretti	Par. 4.11.1 To repeal lines 1047-1048, whereas "For a number of active substances, more than one ADI or ARfD has been established".
152	4.11.1. Existing databases for TTC - non-cancer endpoints	RIVM	Section 4.11.1 Pg 37 Ln 1605 – 1620 It should be explained in these paragraphs how the various research groups estimated their exposures from a mg/m3 basis to a mg/person/d basis. For such calculations usually assumptions on breathing volume and absorption from inhalation are used and non-standardised procedures may lead to significant differences between study results. Also it is very questionable if standard procedures (e.g. the ones used in REACH context really provide adequate estimate of systemic exposure. This would also affect the text in lines 1798 – 1808 on page 41.
153	4.11.2. Considerations for route-to-route extrapolation	ECETOC	Page 38 / Lines 1625-1641 It is mentioned 'that portal of entry effects are not covered which may be of reference'. However, the papers of Carthew et al, 2009 (cited elsewhere); Safford, 2008 (cited elsewhere); and Safford et al, 2011 (not cited) should be considered here, as they address TTC values for portal of entry effects for inhalation and skin sensitisation, respectively. Not cited reference: - Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regulatory Toxicology and Pharmacology. 60, 218-224.

154	4.10.1. Dietary intake estimates for TTC	UK Food Standards Agency	Line 1525 "Such methods…" should be clarified, since the above paragraph includes reference to acute risk assessments being conducted by some Panels, and I don't think it is the intention to describe these as overly conservative.
155	4.10.2. Duration of exposure	DuPont	Line 1580 The proposal of adapting the TTC for shorter exposure durations has been put forward but the Committee has stated they are not confident about the general applicability. No justification/rationale is provided to support their lack of confidence.A qualifying comment to clarify this position would be helpful.
156	4.11. Routes of exposure other than oral	WWF European Policy Office	Line 1587 Another important aspect of multiple routes of exposure is not only relating to workers but also to consumers. Substances such as DEHP can be used in many applications such as food packaging, printing inks, adhesives and there are more exposure routes for one substances. Even more important, risk assessment should also address the combination effects of different chemicals leading to the same adverse outcomes following combined exposures. The rationale for such an approach is well detailed in the US National Academies' report of 2008 (http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=202508). This highlights the need to develop advanced risk assessment approaches which are transparent and inclusive - the TTC approach seems to be the contrary.
157	4.11. Routes of exposure other than oral	RESEAU ENVIRONNEMENT SANTE	Line 1587 Another important aspect of multiple routes of exposure is not only relating to workers but also to consumers. Substances such as DEHP can be used in many applications such as food packaging, printing inks, adhesives and there are more exposure routes for one substances. Even more important, risk assessment should also address the combination effects of different chemicals leading to the same adverse outcomes following combined exposures. The rationale for such an approach is well detailed in the US National Academies' report of 2008 (http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=202508). This highlights the need to develop advanced risk assessment approaches which are transparent and inclusive - the TTC approach seems to be the contrary.
158	4.11.1. Existing databases for TTC - non-cancer endpoints	DuPont	Line 1605-1619 No followup to rationalise the different thresholds proposed by Carthew et al vs Escher et al. Would be useful to explore the reasons behind these discrepancies before concluding on the utility of TTC for inhalation as the route of exposure.

159	4.11.2. Considerations for route-to-route extrapolation	ILSI Europe aisbl	1625-1641 Reference is made to the fact that the TTC does not apply to portal of entry effects. First, we would like to point out that entry effects in the gut would be covered by the oral database. Second, there are other publications that do discuss TTC-based approaches for portal of entry effects for the skin (sensitisation) and lung; for example, see Carthew et al. (2009; referenced elsewhere in the text) and Safford et al. (2008; referenced elsewhere in the text, and 2011; see below). In these publications, TTC values for portal of entry effects for inhalation and skin sensitisation (respectively) are addressed. Safford, R.J., Aptula, A.O. and Gilmour, N. (2011) Refinement of the Dermal Sensitisation Threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regulatory Toxicology and Pharmacology 60, 218–224
160	4.11.2. Considerations for route-to-route extrapolation	Unilever	Section 4.11.2.1 Line No.s 1625-1641 Reference is made to the fact that the TTC does not apply to portal of entry effects. However, this section does not recognise the publications of Carthew et al (2009; referenced elsewhere in the text) and Safford et al (2008; referenced elsewhere in the text, and 2011; not referenced). In these publications, TTC values for portal of entry effects for inhalation and skin sensitisation (respectively) are addressed. This should be added, and the Safford et al (2011) reference included.
161	4.11.2. Considerations for route-to-route extrapolation	DuPont	Line 1751 EFSA 2010d should be cited for clarity rather than just EFSA 2010.
162	4.11.7. Extrapolation route oral to dermal	DuPont	Line 1751 Cited EFSA 2010d document does not propose any refinements to the default parameters. It merely states: - The existing default of 100% in the current Guidance Document (EC, 2004) needs to be revised. - There is no compelling reason to modify the existing default of 10% for substances with LogPow < -1 or > 4 and MW > 500 (EC, 2004). Perhaps this should come across more explicitly in the opinion text.

163	4.11.9. EFSA considerations on route-to-route extrapolation	ILSI Europe aisbl	Lines 1780-1808 Concerning the issues of oral to dermal extrapolation it is recognised that consideration must be given to potential differences between systemic exposure via the two routes. However, a number of points need to be raised here: a. Few robust dermal sub-chronic or chronic toxicity studies have been conducted that would allow the establishment of a database on which to base a dermal TTC. b. Of those conducted, many will not have produced toxicity, and thus the NOAEL will be the highest dose tested, and by definition conservative. This would make any database constructed skewed. c. Extrapolation from oral studies in animals to dermal exposure is commonplace in the risk assessment of dermally applied ingredients in consumer products, taking into account appropriate dermal penetration. This is the recommendation from the SCCS in their notes of guidance (The SCCS"s Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 7th Revision, 2010). In the guidelines, no recommendations are made regarding route-to-route adjustments to account for dermal metabolism. d. While it is recognised that the Kroes et al. (2007) publication does not specifically mention consideration of the oral bioavailability of the chemicals in the Munro database, this database consisted of low molecular weight organic chemicals that are likely associated with extensive oral uptake. Hence, Kroes et al. (2007) focused on the more challenging issue of first pass metabolism. Although a more thorough evaluation of the estimated oral uptake of the chemicals in the Munro database may increase the confidence that can be placed in applying the current TTC limits to dermal exposures, it can be reasonably anticipated that such an analysis would support the assumption that these compounds are well absorbed following oral administration. e. The suggestion that the oral TTC values could be used if the dermal absorption was known from experimental data to be low (e.g. 10% or lower) (line 1793) seems rather arbitrary and is not clearly explained. It seems to imply that this would allow for the possibility that the oral toxicity data might underestimate dermal toxicity for the compounds in the oral (Munro) database by a factor of 10-fold. f. An Expert Group has recently been established within ILSI Europe under project COSMOS (jointly funded by the European Commission through the 7th Framework Programme and COLIPA) to address the issue of oral to dermal extrapolation within the TTC. This expert group is expected to complete their work in two years.

164	4.11.9. EFSA considerations on route-to-route extrapolation	Unilever	Section 4.11.9 Line 1780-1808 Concerning the issues of oral to dermal extrapolation it is recognised that consideration must be given to potential differences between systemic exposure via the two routes. However, a number of points need to be raised here: a. Few dermal sub-chronic or chronic toxicity studies have been conducted that would allow the establishment of a database on which to base a dermal TTC. b. Of those conducted, many will not have not produced toxicity, and thus the NOAEL will be the highest does tested, and by definition conservative. This would make any database constructed skewed. c. Extrapolation from oral studies in animals to dermal exposure is commonplace in the risk assessment of dermally applied ingredients in consumer products, taking into account appropriate dermal penetration. This is the recommendation from the SCCP in their notes of guidance (The SCCS"s Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 7th Revision, 2010). In the guidelines, no recommendations are made regarding route-to-route adjustments to account for dermal metabolism. d. An Expert Group has recently been established within ILSI Europe under project COSMOS (jointly funded by the European Commission through the 7th Framework Programme and COLIPA) to address the issue of oral to dermal extrapolation within the TTC. This expert group is expected to complete in two years.
165	4.11.9. EFSA considerations on route-to-route extrapolation	DuPont	Line 1781 Appears to suggest strong arguments for applying the same TTC approach when dermal is the route of exposure but then concludes that a separate database is required. This comes across as confusing to the end reader.
166	4.12 Potential for application of the TTC concept in the different EFSA Panels	R.I.S.K. Consultancy	EFSA's draft document sometimes imply that a TTC's chief purpose is to be a screen that informs further toxicity testing—just as S-AR has always been. But a TTC is actually a complete substitute for toxicity tests, and its proponents often argue to broaden that use. Regulatory agencies may protest that they will carefully restrict use of the TTC to either screen chemicals for toxicity tests, or restrict its use to large groups of chemicals without test data (its original rationale) But the mere availability of a "safe dose" with a scientific veneer creates in regulators an incentive to substitute this simple number for expensive & complex tests; given also the overwhelming number of untested chemicals, and budgetary limits. Agencies may also protest that they will obey existing mandates to ensure toxicity tests instead of using the TTC. But why are they considering (see following) the TTC in just such situations? If a chemical has insufficient toxicity data, there is no reliable way to know its toxicity, so a TTC is an ill-informed guess as to toxicity. Conversely, if there is enough information to indicate which TTC is appropriate, then there is toxicity data on which to assess its risk (or at least inform how to do the next toxicity test)...and so no need to employ the guess that is a TTC.
167	4.12.1. ANS Panel	DuPont	Line 1813 Typo responsibility has only 1 "l".

168	4.12.1. ANS Panel	Unilever	Section 4.12.1 Line 1811-1815 The Committee concludes that the TTC should not be used for food additives and nutrients. This is purely based on the fact that current regulations require detailed toxicity testing for such ingredients. There is no scientific basis for excluding such ingredients from the TTC scheme. This should be made clear in the document. There is also no reason why the TTC should not be incorporated into the regulation of these ingredients in the future, thus reducing the requirement to conduct animal testing where use levels are suitably low.
169	4.12.1. ANS Panel	ILSI Europe aisbl	Lines 1811-1815 The Committee concludes that the TTC should not be used for food additives and nutrients based on the fact that current regulations require detailed toxicity testing for such ingredients. There is no scientific basis for excluding such ingredients from the TTC scheme. This should be made clear in the document. There is also no reason why the TTC should not be incorporated into the regulation of these ingredients in the future, thus reducing the requirement to conduct animal testing where use levels are suitably low.
170	4.12.2. CEF Panel	INRAN, Rome	As very clearly stated in the opinion "For application of the TTC approach it is essential to have suitably conservative exposure assessments, which take account of high exposure scenarios. It requires information on known or predicted human exposures, for which there is confidence that they are not an underestimate" (line 1906-1909). Since the conservativeness of the scenario used for food contact material is not ensured for all population groups (see comments to paragraph 4.10.1), the SC could mention in its opinion that the exposure scenario used in the CEF Panel is currently under revision so that it is conservative in all population groups. Such revision is needed before the TTC approach can be applied. The illustration by SC of the TTC approach as used in the CEF Panel is related to the technique used for flavouring substances that were already on the market and for which there was the need to conclude safety assessments within a reasonable time frame. For new substances a more stringent and scientific-based approach has been developed by the CEF Panel and the SC opinion should necessarily refer to such new approach when making reference to the opinion EFSA, 2010e: Guidance on the data required for the risk assessment of flavourings to be used in or on foods. European Food Safety Authority. The EFSA Journal 8(6): Available at: http://www.efsa.europa.eu/en/efsajournal/doc/1623.pdf In particular it is crucial to report that, as clearly stated in the opinion, "For any new flavouring substance its genotoxic potential has to be assessed in the first step of the evaluation. This assessment should start with in vitro tests, covering all three genetic endpoints, i.e. gene mutations, structural and numerical chromosomal aberrations." This way of dealing with genotoxicity should be considered by the SC. At its first step it does not include any animal testing.
171	4.12.2. CEF Panel	ANSES	Food Contact Materials: We would not recommend the use of the TTC concept in the case of compounds voluntary added in materials by industry. TTC should only apply to impurities and break-down products with no alert chemical structure.
172	4.12.3. CONTAM Panel	Federal Office of Public Health	1832ff: There are many other situations in which the TTC concept can be used. For instance, after a chemical accident, the soil is polluted, vegetables grown on the field will be contaminated later and there are no toxicity data available for this contaminant.

173	4.12.4. FEEDAP Panel	none	For feed additives, there is no need to apply a TTC approach to genotoxic carcinogens. If there is a structural alert, there will be a requirement to test for genotoxicity. Furthermore, testing for genotoxicity will routinely be required for most substances even if there is no structural alert. If absence of genotoxicity cannot be demonstrated the substance should not be used in animal feed for food-producing animals or for pets as there would be an unacceptable hazard to consumers and to people handling the animals. Although it is not envisioned that FEEDAP will use the TTC approach to identify safe levels of use of genotoxic feed additives, the TTC approach might be a useful tool to use when considering specifications giving limits for the possible presence of genotoxic contaminants in feed additives.
174	4.12.5. PPR Panel	BASF SE	Page 42f, line 1860 ff In the chapter 4.12 the potential application of the TTC concept in the different EFSA panels is summarized. Under the last point PPR panel it is mentioned, that the TTC approach is being actively considered by the PPR Panel for toxicological relevance of plant metabolites and degradates of pesticide active substances. It would be worth to highlight, that the respective SANCO Guidance Document 221/2000 rev. 10 containing the old generic TTC value of 1.5 µg/person/day as one of the trigger values for non-genotoxic non-relevant metabolites of plant protection products, would have to be updated, by using the Cramer class TTC values.

175	Conclusions and Recommendations	ILSI Europe aisbl	Lines 1906-1912 Is it not true for any risk assessment - also when substance specific data are available - that suitable exposure information needs to be at hand? Would the Scientific Committee tend to apply more scrutiny on the exposure side of a risk assessment when the dose-response side were covered by the TTC concept? If so, what would be the rationale? Lines 1923-1929 While it is clear there are opportunities to refine the TTCs and the Cramer classes in particular using current knowledge and the larger databases currently available, there have been no analyses suggesting that the current TTC tiers, including Cramer Class II, are not protective. The proposed conclusion to assess Cramer class II substances based on the threshold derived for Cramer class III substances is quite drastic, has complex consequences, and should be discussed and justified in detail in the opinion text. Should this proposal be implemented for EFSA evaluations, the panel should also comment on what approach to use for flavoring substances, and why. Interestingly, the analysis of Munro et al. 2008 revealed that when separating the organophosphate and organohalogen data from the Munro/Cramer class III data, the Class III threshold becomes similar to the Class II threshold. It is our opinion that removal of the Cramer class II or any similar consolidation of structural classes would be best achieved as part of any systemic review of the whole TTC approach using current databases, rather than limiting parts of the current system. Lines 1935-1942 Looking forward, a scientifically rigorous re-evaluation of the Cramer classes would be part of any update of the TTC approach, particularly looking at the non-cancer levels which may result in different threshold values. In the meantime, since the Kroes et al (2004) tiered TTC approach has an established tier for the OPs at 18 ug/day, these chemicals would not be evaluated as Cramer Class III and thus should not factor into the calculation of new/updated 5th percentile of the distribution of NOAELS in this class. In light of the fact that Munro et al. (2008) have already analysed the distribution for Cramer Class III without the OPs, we feel there is scientific support to set the TTC for Cramer Class III at 180 ug/day. The opinion document should discuss this aspect and provide a rationale for the recommendation. If EFSA prefers to maintain the 90 ug/day as an initial tier for Cramer Class III for pragmatic reasons, we recommend that the text be expanded to indicate that if it is confirmed that the chemical is not in a class known to be an AChE inhibitor, then a higher TTC limit of 180 ug/d is supported by the existing literature. Lines 1971-1975 While there may not be a general agreement on how the prediction of metabolites may be accomplished, it is important to note that there are methods available, and that some measure of metabolic prediction, even if done simply with expert judgment, is necessary to account for the potential of toxic metabolite formation. And as noted in the text there are commercial programs available to predict metabolism. In addition, some structural alert programs such as DEREK and ToxTree do consider key metabolic pathways in their analyses. For example, both programs integrate knowledge of metabolism in the structural alerts for common/known metabolic transformations that produce genotoxic metabolites, such as aromatic amines and diaryl azo compounds.

176	Conclusions and Recommendations	RESEAU ENVIRONNEMENT SANTE	Line 1901: This statement is misleading. The TTC approach cannot be used outside its original scope (priority setting) without losing scientific reliability. Cramer et al 1978 clearly stated that their tool can give a "preliminary assessment of probable risk", necessary for prioritizing which substances need an in-depth toxicological analysis. Substance- specific risk assessment cannot be replaced by application of TTC because of its intrinsic limitations. Line 1904: We recommend the following wording: "The TTC approach should not be used for substances when there is a legislative requirement for submission of toxicity data or where there is the need for a detailed risk assessment." Line 1920: The statement that application of the Cramer classification scheme in TTC is conservative and therefore protective of human health is scientifically not justified. The TTC approach pretends to deliver thresholds for toxicological concern, but the concern for many substances does in fact already start at much lower concentrations (for example, PFOA, nonylphenol and certain phthalates). Line 1933: This statement is not in line with scientific findings on adverse effects of low dose exposures. Line 1961: This conclusion is not convincing as the scientific evidence discussed in the respective contains may gaps and omissions of relevant information. Scientific evidence for non-monotonic dose response curves and new insights into the consequences of prenatal exposures are completely ignored. Line 1997 and 2000 ff: For exactly those reasons as described in the report the risks for the unborn child and pregnant women cannot be assessed by the TTC approach. Line 2023: Proposed exclusions. The TTC approach should never be applied to synthetic substances which are intentionally used. For these substances a chemical safety assessment and substance-specific toxicity data are required. Line 2036: Proposed applications. Many of the groups of contaminants mentioned relate to synthetic substances which are used for a certain purpose. The TTC approach should not be applied to substances used intentionally for FCM, feed additives, flavouring substances, trace contaminants resulting from such applications. Line 2059: Please add the following recommendations: - It should be made clear, that the TTC approach cannot replace submission of substance-specific toxicity data. - It should be made clear, that the TCC approach can be used for priority setting if a large number of substances has to be evaluated. First priority should be to avoid such a situation by reducing the number of contaminants in the environment of the general public. - It should be clearly stated that application of TTC approach cannot ensure that exposures are below critical values. The TTC approach is not conservative. - If threshold values for priority setting are required, they should be based on the lowest available toxicity data.

177	Conclusions and Recommendations	R.I.S.K. Consultancy	PLease explain why, if a TTC is supposed to be as protective as a NOEL times UF (i.e. lower than the claimed dose that has no toxic effect), the TTCs allow roughly 5% of the chemicals to have toxicity at doses lower than this alleged protective TTC.
178	Conclusions and Recommendations	Karolinska Institutet	In general we think that this a clearly written document, which in a balanced way describes the usefulness of TTC approach and at the same time describes its weaknesses and limitations. Generally it also clearly points out under which circumstances the TTC approach should not be used. Exposure situations for which TTC concept may be appropriate and those for which it may not be are discussed. We agree that it is not appropriate to recommend TTC values for inhalation. Arguments for this statement are clearly given throughout the document: difficulties to generate overall exposure estimates for different scenarios, no complete TTC databases for inhalation exposure exist, etc. In addition, suitability of the TTC has not been evaluated for the inhalation exposure. For application of the TCC values to infants and children toxicokinetic is considered and it is recommended that TTC values are converted to corresponding values that take into account body weight. However, different sensitivity for e. g. genotoxic carcinogens have been described at different age periods (see Hattis et al. Environmental Health Perspectives, 2005, 113, 509-). The conclusion of that work is that early-life exposure could make important contributions to full-life cancer risks and that different factors such as differences in DNA-repair contribute. This should be considered before applying TTC values to infants and children. In recommendations for future work nothing is mentioned about research needs considering different carcinogenic mode of actions (MOA). In TCC descision tree chemicals are classified as genotoxic and non-genotoxic. This is clearly an oversimplification. In addition to relatively well defined, genotoxic and nongenotoxic MOAs, many other less well defined MOAs for carcinogens exists e.g. chemicals acting by increasing cell proliferation. To specify these and to identify new MOAs, research is needed. Research about different MOAs for carcinogens should be included in the section of recommendations for future work.
179	Conclusions and Recommendations	Office Risk Assessment VWA	The experts of the Office for Risk Assessment support the EFSA opinion of the TTC as written in the Draft of 12/07/2011. We have no comments regarding the text. We are however left with a question that is not addressed in the EFSA Opinion. To our opinion, the concept of TTC must be implemented in the common approach of the valuation of toxicity of chemicals. Risk assessment bodies, both public and private, must share the concept and there should be an uniform procedure when and how to use the TTC. This procedure should be preferably supported by international bodies such as the WHO. Our question is therefore how the use and implementation of the TTC concept will be organized? We would plea for a next step c.q. project supervised by EFSA for the implementation, with the participation of risk assessment bodies of the members states of the EC.
180	Conclusions and Recommendations	ANSES	Mixtures: TTC approach is not to be used in the case of mixtures. Mixture (or cocktail) effects are, as EDs, one of the challenges risk assessors will have (or already have) to face. Consequently, in the recommendation section, this could appear as a research need.

181	Conclusions and Recommendations	ANSES	Natural toxins: Efsa did not state if TTC concept is applicable to marine biotoxins or cyanotoxins.
182	Conclusions and Recommendations	ANSES	The decision tree EFSA is proposing (page 47) should appear in the summary since it is self-explanatory & summarizes the overall approach EFSA is recommending.
183	Conclusions and Recommendations	Confederazione Nazionale Coldiretti	EFSA SC should consider also the recently released document on Statistical Significance and Biological Relevance (2011), Chapter 3.1., and the reccomendations made on caution to be used for false negatives (absence of evidence). The Decision tree for toxicological screening as provided in TTC Document (p. 14) seems overly prone to false negative endopints. False negative are increased in probability in case of massive immission on the market of new, untested substances. Further reflection could be needed in this area.
184	Conclusions and Recommendations	Confederazione Nazionale Coldiretti	References Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic (Request No EFSA-Q-2004 020) http://www.efsa.europa.eu/en/efsajournal/doc/282.pdf EFSA Scientific Committee; Statistical Significance and Biological Relevance. EFSA Journal 2011; 9(9):2372 doi: 10,2903/j.efsa2011.2372 US EPA (US Environmental Protection Agency) (2005) Proposed guidelines for carcinogenic risk assessment. Federal Register, http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDFUS US EPA(2000) Benchmark Dose Technical Guidance Document http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=22506#Downlod Ronald D. Snydera, , Mark D. Smithb (2005)Computational prediction of genotoxicity: room for improvement. Drug Discovery Today Volume 10, Issue 16, 15 August 2005, Pages 1119-1124
185	Conclusions and Recommendations	Kantonales Labor Zürich	Our comment, part 1 of 5. The Dokument with the tables and a spreadsheet have been sent otherwise: Comment on the public consultation of the EFSA on the concept of the Threshold of Toxicological Concern (TTC) Gregor McCombie, Koni Grob, Christoph Buergi, Kantonales Labor Zurich, Switzerland Summary

			The draft opinion on the TTC concept thoroughly deals with the classification of substances and the TTC values. It also carefully checked updates for the basically old data-sets. However it did not adequately validate the concept in describing its usefulness and applicability: the Scientific Committee was requested to "evaluate the relevance and reliability of the TTC approach" and "advise on the application of the TTC concept" (Terms of Reference). The vague description of the application in the "Conclusions and Recommendations" (line 1901ff) is bound to cause difficulties. EFSA should either completely stay out of risk management, which would mean replacing the relevant paragraph by stating who should specify the application of the TTC concept. Alternatively, EFSA should make more specific recommendations on the applications for which it considers the TTC concept adequate. The concept stipulates that the TTC values provide safety in 95 % of the applications. The opinion should interpret this, e.g. by testing the TTC concept for the list of substances evaluated for FCMs. We noticed cases of potentially severely underestimated risk, but also conflicts by the tolerance derived from the TTC concept being far higher than the existing SMLs. Introduction There is a need for defining a concentration below which substances no longer need to be assessed. The currently often applied "non detection limit" of 0.01 mg/kg food (sometimes 0.02 mg/kg in legislation) is not satisfactory, as it is too high for certain substances, but too low for others. It seems logical to specify a threshold based on the tolerable intake of a substance in dependence of the structure and exposure. So far the draft opinion of the EFSA is highly welcome as a step in this direction. We do not want to comment on the evaluation of the TTC values, but on the massive effect the concept is likely to have once it is sanctioned by EFSA as the leading authority. Unclear application In the Terms of Reference, the Scientific Committee was requested to "Evaluate the relevance and reliability of the TTC concept for application in the food and feed area" and "advise on the application of the TTC concept in areas of chemical risk assessment". Apart from quantifying the reliability at 95%, the only substantial statement found in the draft opinion is in the Conclusions and Recommendations (line 1901ff) and specifies the field of application as "The TTC approach is a useful screening tool for both qualitative risk assessment and priority setting that enables efficient use of available resources and potential reductions in animal testing". This does not specify well enough what should be understood by "useful" and “screening tool for priority setting”, but suggests that animal testing can be reduced (without specification in which cases).
186	Conclusions and Recommendations	ECETOC	Page 44 / Lines 1923 – 1929 and Page 45 /Lines 1943 – 1946 A further sub-division of the Cramer classes may indeed not improve applicability of the TTC concept. However, a re-evaluation of the underlying data sources in combination with newer data should be considered in order to validate and possibly refine the Cramer classes, in particular class II, as the database underlying the latter is limited. Page 46 / Lines 2006 - 2012 For the dermal exposure route, Safford, 2008 (cited) and Safford et al, 2011 (not cited) evaluated LLNA data and proposed a Dermal Sensitisation Threshold level. Keller et al, 2009 (not cited) evaluated human skin data on fragrance ingredients and chemically related substances (e.g. plant extracts or flavours) to derive proposed values for a threshold of sensitisation concern. Thus, these levels were derived from toxicity data based on dermal exposure. Not cited references: - Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regulatory Toxicology and Pharmacology. 60, 218-224. - Keller, D., Krauledat, M., Scheel, J., 2009. Feasibility study to support a threshold of sensitisation concern concept in risk assessment based on human

			data. Archives of Toxicology. 83, 12, 1049-1060. An improvement of the TTC concept for inhalation exposure is currently subject of a LRI-project run at the Fraunhofer institute. It builds on the data evaluation published by Escher et al, 2010 (cited). Further to this, TTC values based on toxicological studies upon inhalation exposure were also proposed by Carthew et al, 2009 (cited); Grant et al, 2007 (not cited); and Bernauer et al, 2008 (cited). It may be worthwhile to carry out a combined evaluation of the underlying data sources. Not cited reference: - Grant, R.L., Kadlubar, B.J., Erraguntla, N.K., Honeycutt, M., 2007. Evaluation of acute inhalation toxicity for chemicals with limited toxicity information. Regulatory Toxicology and Pharmacology. 47, 261-273.
187	Conclusions and Recommendations	ILSI Europe aisbl	Lines 1976-1983: While the Kroes et al. 2004 decision tree does allow progression to the "Cramer class" tiers based on a lack of structural alerts, the tier is used or discussed by other work on threshold levels, especially in regions where carcinogens are mostly assessed by default with linear low dose extrapolation. We suggest therefore to reword along the lines of: 'The original FDA Threshold of Regulation value of 1.5 µg/person per day is of historical importance, but has little practical application in the overall TTC approach for systemic chronic effects via the oral route of exposure as presented in Figure 2.'

188	Conclusions and Recommendations	Kantonales Labor Zürich	Our comment, part 2 of 5 It seems clear that human exposure to substances in amounts in excess of the TTC values should be treated with first priority. The problem, however, is the specification of a second priority: What is meant to happen with unevaluated substances the exposure of which is below the TTC value? In the field of food contact materials (FCMs), one of the potential main application areas of the TTC concept, it is foreseeable that the sanctioning of the TTC concept by EFSA will be seen as a discharge of any toxicological testing of substances for which the human exposure can be shown to be below the TTC. For enforcement it will be impossible to demand further safety evaluation, which means that the TTC values effectively become equivalent to legal limits. Since the large majority of the substances migrating into food are not specifically regulated, the TTC concept as it stands is likely to trigger a fundamental change in FCM regulation. In view of this potentially radical impact, EFSA should not cover the field of application by a sentence as vague as cited above. In our opinion, it should either completely stay out of advising industry and management and specifically delegate this task to other bodies, or provide better guidance. Completely staying out would mean that the paragraph in the "Conclusions and Recommendations" (line 1901 ff) is eliminated and replaced by stating who should specify the application of the TTC concept. This is not really satisfactory, however, since one of the principal tasks in the Terms of Reference was the applicability and reliability of the concept and advising on the application. Alternatively, EFSA should advise industry and authorities on the application of the TTC concept, which primarily means the evaluation of the uncertainty in the classification and the TTC values as well as the interpretation of this uncertainty for industry and managing authorities. In particular it should be clarified what needs to be done if exposure to an unevaluated substance is below the TTC. Does EFSA say that 95 % safety is sufficient in all applications (which would have strong implications for previous evaluations by SCF/EFSA as well as present legislation)? Is it sufficient for some situations, but not for others (to be specified)? Illustration of uncertainty by the set of substances evaluated for FCM EFSA states that the TTC values are sufficiently conservative for 95 % of the substances. This might sound high, but it means that for every one hundred randomly selected compounds, a handful of substances will be assessed to be less toxic than they are. Considering the many thousand compounds that migrate from FCMs into food, there are a considerable number for which the health risks would be underestimated. In our opinion EFSA could provide a fairly realistic picture of the uncertainty by using the set of monomers and additives in the Plastic regulation (PIM, 10/2011) for which SCF and EFSA have provided evaluations. ...
189	Conclusions and Recommendations	Kantonales Labor Zürich	Our comment, part 3 of 5. The Dokument with the tables and a spreadsheet have been sent otherwise: ... Our own attempt We performed such an analysis for substances in Annex I of the Plastic Regulation 10/2011, pretending that there were no toxicological data available and all compounds needed to be assessed by the TTC concept. The raw data is in the separate Excel spreadsheet. Annex I contains 885 monomers and additives, many of which have specific migration limits (SMLs). In order to automate the assessment with the Toxtree software, we used structure files found for 529 of these substances (many of the others are mixtures). Using the batch analysis mode, we ran the Benigni/Bossa rulebase for mutagenicity and carcinogenicity, the Kroes TTC decision tree and the Cramer rules with extensions. Substances with at least one of the following results were excluded from further analysis:

			1. Substances that gave results other than "negative for genotoxic carcinogenicity" AND "negative for nongenotoxic carcinogenicity" by the Benigni/Bossa rulebase for mutagenicity and carcinogenicity. 2. Substances that did not give the result "substance would not be expected to be a safety concern" by the Kroes TTC decision tree. The full results for all substances in the PIM can be found in the Excel attachment. We identified three critical cases. Case 1: The toxicologically derived SML is lower than the TTC value (Table 1) As expected, most SMLs are above the TTC values, which confirms that the TTC concept is mostly conservative. However, for 4 substances listed in Table 1, the TTC value is higher than the SML: 2-(4-dodecylphenyl)indole; phthalic acid dibutyl ester (DBP); 1,4-dihydroxybenzene; phthalic acid bis(2-ethylhexyl) ester (DEHP). The SML for phthalic acid dibutyl ester is 0.3 mg/kg. This substance is classified as Cramer I, which would let us believe that a daily intake of 1.8 mg is safe, i.e. that a concentration of 1.8 mg/kg is tolerable for an assumed daily exposure by 1 kg food. For the SML, an allocation factor of 2 was assumed owing to other sources of exposure, but this does not match the difference. The assumed food consumption has a more important impact on the concentration of chemicals tolerated in food. With current regulation this is set at the non-negotiable level of 1 kg per day, but in reality, most often clearly less than one 1 kg of food consumed per day contains the given substance. Then the maximum concentration in food that corresponds to the TTC value is rather often above the SML: - For 22 substances listed in Table 1, the concentration that can be tolerated in food derived from the TTC value is higher than the SML if a daily consumption of 100 g food containing the substance is assumed. - For 35 substances listed in Table 1 (of the 258 that were analysed and had an SML or SML(T)), the concentration that can be tolerated in food derived from the TTC value is higher than the SML if a daily consumption of 20 g food containing the substance is assumed. ...
190	Conclusions and Recommendations	none	Conclusion ff. is based on estimating the risk from genotoxic carcinogens by use of linear extrapolation from the high doses used in animal experiments to the low levels of exposure of human consumers. Such an approach is not normally accepted in the EU. Furthermore, not all substances give an estimate of acceptable carcinogenic risk at the proposed TTC for genotoxicants. This is dealt with by excluding those known classes of substances that have an estimated risk that is unacceptably high at the propose TTC. I have concerns that some other substances might also have high cancer risks at the TTC level of exposure. The assumption that substances will not be particularly potent carcinogens seems to be a major loophole in the application of this level of TTC to genotoxic carcinogens. It seems unreasonable to include an assumption about toxicological potency in a method designed to identify a level of exposure with essentially no toxicological effect.

191	Conclusions and Recommendations	FEFANA	Lines 2076-2077: EFSA recommends to add new toxicity data expressed as BMDL"s rather than NOAEL/LOAEL"s and to express the potency in terms of molar equivalents instead of mass per body weight. This recommendation is not supported by any sort of explanation. Therefore FEFANA has doubts about the related advantages notably for the molar equivalent since most of the present databases are expressed in mass per body weight and not as molar equivalents. FEFANA would also appreciate if the advantages of the BMDL approach would be better described in the document; taking also into account that we think the NOAEL/LOAEL"s approach is more conservative.
192	Conclusions and Recommendations	PAN Europe	Summary: TTC (threshold of toxicological concern) is a fixed figure of human exposure and if the exposure of a certain chemical is below this figure/threshold, the chemical is classified as “safe” and no further testing is needed. Most chemicals are placed in a ‘class’ called Cramer III and EFSA assumes a level of 90 µg is safe for daily intake of adults, lifelong. Some (smaller) groups have a higher TTC, some lower (nerve poison chemicals 18 µg/day, genotoxic carcinogens 9 µg/day). TTC is used already for flavouring chemicals, is now proposed by EFSA for chemical impurities and metabolites which industry is reluctant to test, but no doubt industry is and will keep on pushing to extend TTC to all chemicals like those in REACH. TTC is constructed to ease the access of chemicals to the market and puts people cumulatively at increasing risks at every step of its design: 1. The TTC uses regulatory (very) old narrow-focussed industry study data (NOEL’s, no effect level) as a basis; EFSA did not check the original studies because there were non-retrievable; 2. TTC accepts harm by using a cut-off level (5th percentile) in stead of the lowest available NOEL, allowing adverse effects of exactly the most toxic group of chemicals; 3. TTC disregards independent scientific research on chemicals and the much lower data available to falsify these alleged industry “no-effect” data 4. TTC disregards chemical mixtures to which humans are exposed 5. TTC ignores effects on vulnerable groups like foetus and infants 6. TTC allows lifetime human exposure likely calculating/estimating average intake masking peak/acute doses 7. TTC disregards decades of scientific progress ao. on vulnerable windows of exposure during development with epigenetic processes, EFSA even includes endocrine disrupting chemicals; TTC once was intended and used only to get an indication of risk; now its many proponents have crossed a line of justifiability saying it guarantees safety, a claim which is not based on science but on statistical juggling and old unverifiable data which are false;

193	Conclusions and Recommendations	PAN Europe	I. The TTC construction is not applicable for risk management. (first part) - TTC is an obvious manipulation of the industry database by using a cut-off level of 5th percentile of the data instead of using the lowest data; pesticide Dieldrin is 30x more toxic than the NOEL were the "safe level" of TTC is based upon. TTC this way removes largely the uncertainty factors of vulnerability, risk assessment policy used for decades. - TTC is scientifically flawed because it allows one chemical to occupy the complete 'pollution space' in human food (3 kg of food is allowed to be fully polluted by one chemical to the TTC level) completely ignoring the exposure of humans to hundreds of other harmful chemicals every day; - Infants are not protected by TTC. Development causes far greater numbers of genes to be active, and in a much more complicates fashion than adults; a process very vulnerable to insult. EFSA acknowledges infants are more vulnerable, having not only less capacity of renal clearance and less detoxifying enzymes but also has more food intake and different diets, estimated by EFSA to be 2-5x more vulnerable. EFSA though assumes –based on no science- there is an opportunity to use TTC, saying: "careful consideration needs to be given to whether the TTC approach should still be applied"; - Even industry (Kroes, 2004) concludes TTC cannot be used for endocrine disrupting chemicals (EDC's) because of the uncertainty of low-dose effects; EFSA however assumes TTC is safe for EDC's. A quick scan by PAN Europe of research data (Table below) shows industry is right and EFSA wrong; Chemical (Endocrine disruptor /Cramer III Independent studies NOEL/LOEL* (findings in µg/kg bw.) that are lower than 'safe' TTC-NOEL of 150 µg/kg Bisphenol A 2 (LOEL, mice**) Fenarimol 2 (LOEL, mice) DES 0,02 (LOEL, mice**) Atrazin 1 (LOEL, mice) PBDE-99 60 (LOEL, rats) Nonylphenol 100 (LOEL,rats) Di-n-butyl phthalate 10 (LOEL, rats) Hexachlorobenzene + 123-trichlorobenzene 0,1 (LOEL, rats) BDE-47 2 (LOEL, rats)

			0,2 (LOEL, lambs) Perchlorate 10 (LOEL, rats) Methoxychlor 20 (LOEL, mice) Octylphenol 10 (LOEL, pigs) Deltamethrin 3 (LOEL, rats) 0,p'-DDT 18 (LOEL, mice) PFOA 10 (LOEL, mice) Ethinylestradiol 0,2 (LOEL, rats) (*) NOEL is no observed effect level, LOEL is lowest observed effect level (**) from Melnick 2002 EFSA ignores effects of endocrine disrupting chemicals which are observed at a 10, 100, 1000 or even in one case 7500x lower dose than the TTC. Using the TTC for instance for atrazin would mean a daily acceptable intake of 1,5 µg/kg bw. day is acceptable, while at 1 µg/kg effects are already visible in mice; and the extra vulnerability of infants is even not calculated in this case.
194	Conclusions and Recommendations	UK Food Standards Agency	Line 1905 Conclusion w: It needs to be more clear when the TTC should be applied – i.e. not when there are existing relevant toxicity data. At present it looks as though it is intended to be used in all circumstance except when there is a legislative requirement for toxicity testing.
195	Conclusions and Recommendations	UK Food Standards Agency	Lines 2000-2004 Conclusion ll: This recommends applying to infants < 6 months on a case-by-case basis. This has implications for the ADI/TDI, which so far have been assumed applicable to > 3 or 4 months. How does the SC propose to address this issue?

196	Conclusions and Recommendations	UK Food Standards Agency	Line 2026 The term “aflatoxin-like” carcinogens is not meaningful. It can only mean aflatoxins themselves, and you would not use a TTC approach since there are animal and epidemiological data available. Lines 2050-2051 Conclusion rr: This does not appear to be founded on any discussion in the document. Lines 2075-2077 Recommendation f: This does not appear to be founded on any discussion in the document.
197	Conclusions and Recommendations	DSM	line 2076 to 2077: EFSA recommends to add new toxicity data expressed as BMDL"s rather than NOAEL/LOAEL"s and to express the potency in terms of molar equivalents instead of mass per body weight. This recommendation is not supported by any sort of explanation. Therefore we have doubts about the related advantages notably for the molar equivalent since most of the present databases are expressed in mass per body weight and not as molar equivalents. We also would like to know the advantages of the BMDL approach, taking also into account that we think the NOAEL/LOAEL"s approach is more conservative.
198	Conclusions and Recommendations	HESI (ILSI Health and Environmental Sciences Institute)	Summary (lines 525-528) Section 4.4.1.5 (lines 1203-1209) Conclusions and Recommendations (lines 2023-2029) HESI PATC comments on EFSA’s DRAFT Scientific Opinion on exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). The ILSI Health and Environmental Sciences Institute (HESI) Protein Allergenicity Technical Committee (PATC) welcomes the opportunity to comment on the allergenicity aspects of the European Food Safety Authority (EFSA) “DRAFT Scientific Opinion on exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC).” The PATC agrees that the current state of knowledge concerning thresholds of exposure for allergenic proteins does not facilitate use of a TTC. The current literature is not sufficient to establish sensitization and elicitation threshold levels for protein allergens. Aspects of food protein handling, such as the loss of functional activity observed for most proteins during food processing (including proteins currently introduced into GM crops) also bears consideration in the safety assessment of GM crops (Hammond and Jez, 2011); functionally inactive proteins pose a negligible toxicological risk. While a small number of well documented protein toxins display potent oral toxicity, the vast array of dietary proteins are innocuous and provide essential amino acids for nutrition. Highly conservative bioinformatic comparisons (amino-acid homology searches) can identify whether or not introduced proteins expressed in GM crops share meaningful similarities with known protein toxins or allergens. Bioinformatics comparisons are also capable of identifying highly homologous counterparts, which may have shared function and/or may have a history of safe consumption in food. This can help assess the toxicological safety of introduced proteins. Therefore, applying a TTC approach to an introduced protein unrelated to known toxins and allergens may be reasonable and may provide an ethical route to avoid unnecessary animal studies (e.g. 28-day rodent study with protein). References:

			Hammond, B.G., Jez, J.M., 2011. Impact of food processing on the dietary risk assessment for proteins introduced into biotechnology-derived soybean and corn crops. Food and Chemical Toxicology 49, 711-721.
199	Conclusions and Recommendations	Federal Office of Public Health	An updated chapter on how to deal with mixtures of substances of unknown toxicity would be useful as it was described in the monograph by Barlow (2005).
200	Conclusions and Recommendations	(commenting as a private individual)	The Scientific Committee identifies 4 TTC values which it concludes are 'sufficiently robust and conservative' and 'sufficiently protective' for use in EFSA's work. However, it is not clear what level of protection the SC has considered sufficient. On page 19 (lines 794-6) it is stated that the SC 'considers that there is a very low probability of any appreciable cancer risk to human health' from exposures below the TTC of 0.15ug/person per day. However, the meaning of 'appreciable cancer risk' and 'very low probability' is not specified. Since it is envisaged that the TTC may be applied in the future to a large number of chemicals, it would be desirable to have an estimate of what proportion of chemicals might have a 'virtually safe dose' (VSD) below the TTC and how much below the TTC their VSDs might be. These questions could be addressed in terms of margins of exposure, if preferred. Of course the answers would be approximate, so it would be important to characterise the uncertainties affecting them. Some of the information needed for considering these issues is provided on page 19 of the opinion, especially in lines 776-789, but it is very difficult for the reader to make their own judgment from this and therefore difficult for the reader to understand what level of protection the SC has considered sufficient. For non-cancer endpoints, similar questions apply. It is apparent that the TTCs for non-cancer endpoints are based on an estimate of the 5th percentile NOEL, so 5% of chemicals may be expected to have lower NOELs, however it is not explained why the 5th percentile (rather than a lower or higher one) is the appropriate basis for setting the TTC. It would also be useful to have more indication of how much lower the NOELs of this 5% might be. Again, without this information, it is difficult for the reader to understand what level of protection the SC has considered sufficient.

201	Conclusions and Recommendations	TNO	In section w, line 1903-1905 it is stated 'It would not normally be applied when there is a legislative requirement for submission of toxicity data'. By mentioning this prerequisite so explicitly application of the TTC in case of legal data requirements would become impossible. However, the TTC approach could, in our opinion, also be useful in case of evaluations of food components present in low concentration such as reaction products, impurities, break-down products which legally do require a data set. We suggest to revise this part of the conclusions. In section bb, line 1935-1942 it is stated that 'removing such substances from Cramer Class III (which are the most potent substances in that class) might be considered to have an impact on the existing value for Cramer class III. However, pending any future revision of the TTC approach, the Committee concludes that it would be prudent to maintain the value for Cramer Class III at 90 µg/person per day." This conclusion is referring to future revisions of the thresholds. These revisions would increase the threshold for Cramer Class III as was shown by Munro et al 2008 (Toxicology Letters 180, 151–156). According to Munro et al. (2008) 'Removal of both organophosphates and organohalogens from the Cramer class III substances allows a threshold of 600 kg/person/day''. Higher thresholds would extend the applicability of the TTC approach extensively. Therefore, it would be favorable in case these revisions could be made as soon as possible. In section oo, line 2023-2034 it is stated that 'the TTC approach should not be used for the following (categories of) substances: - High potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds). - Inorganic substances - Metals - Proteins - Substances that are known or predicted to bioaccumulate . - Substances with structures that are not adequately represented in the original databases from which the TTC values have been derived, e.g. nanomaterials and radioactive substances. - Substances likely to have the potential for local effects on the gastro-intestinal tract" It is noted that some of these groups were already incorporated in the original TTC decision tree. However, some groups like 'substances known or predicted to accumulate' and 'substances not adequately represented' are newly added. Identification of each and every single peak in a chromatogram is a very laborious if not hardly possible, whereas the health relevance thereof, in case components are present at low exposure, is questionable. Therefore we suggest mentioning specifically those classes of substances (such as dioxins) to which this concerns. We envisage that a practical application of the TTC concept is not possible if this is not specifically mentioned.
203	Conclusions and Recommendations	Federal Public Service Health, Food Chain Safety and Environment	x It is important to compare the correct kind of exposure estimate (chronic versus acute) with a TTC. Maybe the same exposure estimate can not be used for all TTCs when going through the sheme? EFSA could have more attention for this issue. aa, bb, ff, gg: why TTC values are expressed per person, when in kk it is suggested that TTCs should be expressed on a body weight basis? ff EFSA fixes a TTC for genotoxic carcinogens. However, when toxicity data exist, EFSA is not used to derive a health based guidance value but refers to the non-threshold character of toxicity. Are these approaches consistent? How to interpret exposure data if EFSA sets a TTC and no other value in a subsequent non TTC approach assessment?

			oo substances for which already a health based guidance value or BMDL exists, should be excluded from the TTC approach, to avoid that two values (TTC and e.g. ADI) are applicable for the same substance for comparison with exposure estimates oo substances for which authorisation procedures require toxicity testing, could be excluded
204	Conclusions and Recommendations	WWF European Policy Office	Line 1901: This statement is misleading. The TTC approach cannot be used outside its original scope (priority setting) without losing scientific reliability. Cramer et al 1978 clearly stated that their tool can give a "preliminary assessment of probable risk", necessary for prioritizing which substances need an in-depth toxicological analysis. Substance- specific risk assessment cannot be replaced by application of TTC because of its intrinsic limitations (see our detailed comments). Line 1904: We recommend: "The TTC approach should not be used for substances when there is a legislative requirement for submission of toxicity data or where there is the need for a detailed risk assessment." Line 1920: This statement is scientifically not justified. The TTC approach pretends to deliver thresholds for toxicological concern, but the concern for many substances does in fact already start at much lower concentrations (see examples for PFOA, nonylphenol and certain phthalates). Line 1933: This statement is not in line with scientific findings on adverse effects of low dose exposures. Line 1961: This conclusion is not convincing as the scientific evidence discussed in the respective contains may gaps and omissions of relevant information. Scientific evidence for non-monotonic dose response curves and new insights into the consequences of prenatal exposures are completely ignored. Line 1984ff: It is unlikely that a subset of round 500 substances drawn randomly covers the large variety of chemical structures, the high diversity of toxicological endpoints and the differences in vulnerability of for different groups exposed. Line 1997 and 2000 ff: For exactly those reasons as described in the report the risks for the unborn child and pregnant women cannot be assessed by the TTC approach. Line 2023: Proposed exclusions. The TTC approach should never be applied to synthetic substances which are intentionally used. For these substances a chemical safety assessment and substance-specific toxicity data are required. Line 2036: Proposed applications. Many of the groups of contaminants mentioned relate to synthetic substances which are used for a certain purpose. The TTC approach should not be applied to substances used intentionally for FCM, feed additives, flavouring substances, trace contaminants resulting from such applications. Line 2050: It seems to be preferable to achieve this important aim of reducing animal testing rather by using other scientific approaches such as a combination of intelligent testing strategies, increased use and development of non-animal tests as well as higher acceptance of in-vitro regulation and increased use of all available data published in the scientific literature (even if not GLP). Line 2053: generic scheme. This scheme contains many of the flawed assumptions and we would like to refer to our detailed criticism made under the other points. EFSA extends the application from TTC as a priority setting tool to a risk assessment tool, but this cannot be done without leaving the scientific base.

			Line 2059: Please add the following recommendations - It should be made clear, that the TTC approach can not replace submission of substance-specific toxicity data. - It should be made clear, that the TCC approach can be used for priority setting if a large number of substances has to be evaluated. First priority should be to avoid such a situation by reducing the public"s exposures to contaminants. - It should be clearly stated that the TTC approach cannot ensure that exposures are below critical values. The TTC approach is not conservative. - If threshold values for priority setting are required, they should be based on the lowest available toxicity data.
205	Conclusions and Recommendations	DuPont	Line 2061: The outcome was made from the JRC survey that some refinements to clarify the questions could benefit the use of the Cramer. Is it really a recommendation of the Committee that the entire Cramer scheme needs a rewrite?
206	Conclusions and Recommendations	DuPont	Line 2065: The recommendation is unclear- what toxicity groups?
207	Conclusions and Recommendations	DuPont	Line 2072: This recommendation does not follow from the text on in silico tools. The text merely provides a brief overview of the availability of models and then cites the need to consider their domain of applicability when applying them. There is no discussion of what refinements are merited.
208	Conclusions and Recommendations	DuPont	Line 2075: If further substances are added to the databases that currently underpin the TTC approach, it would be desirable to express all toxicity values as BMDLs rather that NOELs and express potency in terms of molar equivalents rather than mass per body weight. Some further clarification in the text is warranted to provide some practical insight of how this could be realised.
209	Conclusions and Recommendations	INRAN, Rome	Line 2054: Figure 2 p. 47 should be modified and “no safety concern” should be changed into “low priority for comprehensive risk assessment” (see comments to lines Line 404-407 in the introduction). It is important to clarify in the text of the SC opinion how the conclusion of an opinion based on the TTC approach would read. A possibility could be ”No risk assessment was deemed necessary for substance X since, based on the TTC approach, there is a low probability of safety concern in relation to its presence in the diet at the estimated level of exposure”. Line 1963-1975: There is no consensus in the scientific community on these SC recommendations. The threshold of 0.15 µg/person per day should only be used when there is a need for urgent release of an opinion on a substance for which no data exist (e.g. a new contaminant). In particular it should not be used for deliberately added substances to food/feed or for substances migrating from FCM for which guidelines require submission of genotoxicity testing data. The approach very recently developed by the CEF Panel to deal with genotoxicity of flavouring substances should be considered (http://www.efsa.europa.eu/en/efsajournal/doc/1623.pdf).

210	Conclusions and Recommendations	Kantonales Labor Zürich	Our comment, part 4 of 5. The Dokument with the tables and a spreadsheet have been sent otherwise: ... Case 2: Presumed carcinogens (SML=ND or 0.02 mg/kg) not recognized as carcinogens (Table 2) The Cramer classification is applicable only if carcinogenicity can be ruled out. There are, however, 16 (from 43) substances with an SML <0.05 mg/kg (mostly "non-detectable", "ND", in one case 0.02 mg/kg) for which the Benigni/Bossa rulebase does not recognize carcinogenicity. Of these substances, 10 are classified as Cramer II or III, which would suggest that 0.09 mg/kg should be considered safe if 1 kg of food containing them is daily consumed; 4.5 mg/kg would be acceptable if exposure is estimated to be only by 20 g food per day. The other 6 substances are classified as Cramer I, which would suggest than 1.8 and 90 mg/kg should be considered safe when the assumed consumption is 1 kg or 20 g per day, respectively. Case 3: SML = 0.05 mg/kg based on negative mutagenicity tests (Table 3) For 69 substances, an SML of 0.05 mg/kg is specified. Presumably experimental results showed absence of mutagenicity, but the toxicological profile was not further investigated. After substantial financial investment, these petitioners were granted the SML of 0.05 mg/kg. If the TTC concept were applied, negative in silico evaluation of carcinogenicity would result in a tolerance of 0.09 mg/kg if the substance is classified as Cramer II or III and 1 kg of food containing the substance is consumed per day, but beyond the overall migration limit for Cramer I substances, if exposure is assumed to be low. Hence, industry not having evaluated migrants from their products in the past may now assume that authorities accept far higher migration after just an in silico evaluation. This may not be the intention of EFSA, but the draft opinion does not provide a hint that this interpretation should be considered inadequate. This application of the TTC concept to the substances evaluated for plastics showed that 16 from 43 substances are not recognized as carcinogens and that for 4 substances the TTC values are higher than the SMLs even for a daily exposure by 1 kg food. For the 529 substances extracted from the Plastic Regulation 10/2011, this is a failure rate of 3.8 %. Among the substances with underestimated health risks are prominent ones, as shown in tables 1-3. Particularly for the substances not recognized as carcinogens, the difference in concentrations considered safe may exceed a factor of 1000 (from the present 0.01 mg/kg to 10 mg/kg for a daily exposure by 180 g food). Within the TTC concept, the consequences of a false negative carcinogen can be even more severe: 1.8 mg per day (Cramer I) instead of 0.15 µg per day (carcinogen). Much depends on the assumptions made for exposure: If producers claim an exposure substantially below 1 kg food per day, for totally 120 of the 529 substances the tolerance through the TTC values could be higher than is acceptable by the SMLs under current legislation.
211	Conclusions and Recommendations	Kantonales Labor Zürich	Our comment, part 5 of 5. The Dokument with the tables and a spreadsheet have been sent otherwise: ... Conclusion In the field of the FCMs, some industries invested large amounts of money into toxicity testing and accepted conservative rules, such as 1 kg of food containing the substance being consumed every day. Other industries cared less about assessing safety and will now deduce from the proposed EFSA opinion that many of their substances are factually sanctioned free of charge and often with far higher tolerance. Since the SCF/EFSA derived the previous rules and evaluated the substances, this draft opinion cannot validate and authorize a new concept without delimiting it against the old rules. The draft opinion provides a validation of the TTC values, but it falls short to "Evaluate the relevance and reliability of the TTC concept for application in the food and feed area" and to "Advise on the application of the TTC concept in areas of chemical risk assessment". The draft should not be published without adding advice on the application of the TTC concept. Annex: Tables as Excel files

212	Conclusions and Recommendations	DuPont	Line 1923 – point z Whilst it is true to say that Class II substances are fewer in number than those in Class I and III, we would urge the Committee to reconsider ignoring Class II completely. Perhaps the implication for food flavourings and metabolic pesticides may not be far reaching though assessing substances on the basis of Class III appears overly conservative. Wouldn't this mean that the TTC and the 3 classes would have to be re-assessed and recombined into 2 classes in order to derive a new threshold. This rather detracts from the evaluation conducted to formulate this Opinion. What impact might such a change have on other areas where TTC could be applied? The justification for treating Class II is only cited on account of few numbers. Some further substantive discussion is needed in the body of the opinion.
213	Conclusions and Recommendations	Unilever	Conclusions and Recommendations Line No.s 1930-1934 It is noted that the Committee conclude that the TTC for Cramer class II materials should not be used, and chemicals falling into this class should be treated as Cramer class III. Although we do not entirely agree with this, we acknowledge that the data set supporting Cramer class II materials is limited.
214	Conclusions and Recommendations	Unilever	Conclusions and Recommendations Line No.s 1935-1942 It is also noted that the Committee conclude that the TTC for Cramer class III materials should remain at 90ug/day despite the recent analysis and publication by Munro et al (2008). Whilst this is prudent, it is considered unnecessary. The analysis conducted was detailed and came from a reputable source. We would think that this would provide enough evidence to raise the TTC to 180ug/day as proposed by Munro. The Munro paper should at least be acknowledged in the text, and referenced.
215	Conclusions and Recommendations	PAN Europe	III. Juggling with data. - TTC is exclusively based on data derived from very old and generally irretrievable industry-sponsored studies. While the reliability of industry-sponsored studies is unknown, EFSA completely relies on these studies and has full trust in the outcome while EFSA never saw the original documents; - The NOEL's , the TTC is based upon, could easily be falsified by PAN Europe using data from independent science (see Table below); whereas the input data from TTC, largely collected by industry lobby club ILSI, are simply wrong. Chemical (Cramer III) Independent studies NOEL/LOEL* (findings in µg/kg bw.) that are lower than 'safe' TTC-NOEL of 150 µg/kg Diquat 100 (LOEL, rats) PFOS 50 (LOEL, male mice) Tetrahydrocannabinol 2 (LOEL, rats) Paraquat 100 (LOEL, mice) Lindane 62,5 (LOEL, rats)

			Flocumafen 80 (LOEL, rats) Toxaphene 50 (LOEL, rats) Cholinesterase substances Independent studies NOEL/LOEL* (findings in µg/kg bw.) that are lower than 'safe' TTC-NOEL of 30 µg/kg Chlorpyrifos 10 (LOEL, rats) (O-ethyl-S-[2(di-isopropylamino)ethyl] methyl phosphonothioate), 2,25 (LOEL, rats) Diisopropylfluorophosphate 10 (LOEL, monkeys) Dimethoate + Malathion 0,4 (LOEL, mice) Methamidophos 2 (LOEL, rats) - It is a shame EFSA –as it did before- disregarded independent literature; - Using the 5th percentile as a cut-off level means the most toxic chemicals are disregarded in TTC-setting. This is based on no science; Dieldrin has a 30-fold lower NOEL and using TTC for it would put humans, let alone infants, at great risks Chemical (Cramer III) Industry NOEL lower than 'safe' TTC-NOEL of 150 µg/kg Avermectin A 30 Dicrotophos 100 Chlordane 55 Dieldrin 5 Dimethoate 50 Disulfoton 50 Fenamiphos 100 Haloxyfop-methyl 50 Hexachlorbenzene 80 Merphos 100

			Methylparathion 25 Quinalphos 30 Sodium fluorate 50 Trenbolone acetate 25 Zeranol 12,5 If one would consider TTC applicable, always the lowest available data should be taken as a basis, consistent with the precautionary principle; - NOEL's anyway are no 'zero-effect' level; it is just the level where in narrow-focussed and insensitive tests no effects are observed; the assumption of a zero-effect is wrong ; - Other organisms, especially waterorganisms, are certainly not safe at TTC levels: Cypermethrin kills waterorganisms at a level >2 billion times lower than the TTC . - The work of EFSA is anyway sloppy given the use of an "independent" dataset (Kalkhof, page 23) of data from 28-day subacute tests with an unsure relation with an unsure relation with chronic data.
216	Conclusions and Recommendations	PAN Europe	IV. Juggling with groupings. - Industry lobby club ILSI started in 1996 (Munro et al.) by juggling with groups of chemicals and data. Short-term toxicity data were artificially upgraded to chronic data. Some genotoxic substances were excluded to prevent an undesirable low TTC, data of dog studies excluded because too many effects were seen, data from food ingredients with extreme high dose NOEL's included in the database to guarantee a high TTC, organophosphates included and later again excluded. Other groups removed like bioaccumulative substances and a classification system based on questions (Cramer) adopted. A highly artificial grouping system resulted with limited relation to reality - The main 'class' (Cramer III) was based solely on company reports which were non-retrievable; - ILSI/Munro database is a unbalanced selection of tests while studies on immunotoxicity were lacking and hardly any on endocrine disruption included;

217	Conclusions and Recommendations	PAN Europe	V. EFSA's blind love of industry lobby club ILSI - EFSA showed its lack of impartiality already in 2005 when a joint meeting was convened with industry and ILSI on genotoxic substances , excluding all other stakeholders. Here already EFSA started embracing industry's effort to undermine the non-threshold approach of the EU and embracing ILSI proposals (MOE, margin of exposure, similar to TTC); - The Scientific Committee on Food in 1996 expressed concern that TTC might not be acceptable for low-dose toxicity of neurotoxic, immunotoxic, endocrinologic and developmental toxic events. EFSA sees no concerns anymore now industry in a study claimed safe use for all categories (Kroes 2000); - Concerns if the TTC approach can be used for infants were addressed by an ILSI-workshop in 1998 (page 31 EFSA consultation) and accepted by EFSA without any scrutiny; - People with very tight connections to ILSI (Boobis, Moretto ao.) have been part of the EFSA panels for many years and even part of this very panel. WHO in contrast restricted ILSI from activities because its track record of putting the interest of its corporate members first . - It is remarkable EFSA with a mission to protect health of EU citizens has such a high -almost blind- confidence in corporate information; latest EU monitor of food risks (2010) shows only 4-5% of the EU citizens are "very confident" with the information of companies; - People heavily involved in industry lobby club ILSI (Kuiper, Boobis) and promoters of the TTC-idea in publications (Barlow, Larsen, Piersma, Schlatter) are part of this very EFSA panel to scrutinise TTC. How on earth can this result in a fair or objective outcome?
218	Conclusions and Recommendations	PAN Europe	I. second part - EFSA states –without any evidence- the two generation test (OECD TG 416) is considered the critical test for hazard assessment of most endocrine parameters and this would mean TTC is safe for endocrines. It is known however –as pointed out by a US-NIEHS panel- this test has severe shortcomings like insensitivity on developing effects, missing endpoints on endocrine disruption, and short follow-up of F2 (Melnick, 2002): The findings of the NIEHS-panel in this study indicate a change of testing paradigm is needed for endocrine disrupting chemicals. [Note EFSA, remarkably, proposed to delete this "critical" two-generation test for pesticides and substitute it with a less informative extended one-generation test (The EFSA Journal (2007) 449, 1 – 60)]. - Scientific progress in the last decades is completely disregarded by basing the TTC on old outdated narrow-focussed studies. It is for instance already well-established that the in utero and perinatal "environment" and maternal and early childhood circumstances play major roles in the risk of later life disease with epigenetic processes .
219	Conclusions and Recommendations	PAN Europe	II. Established EU policy will be undermined on many levels if TTC would be adopted. - If we would do a quick scan of available data and use the very high intake for pesticides (90 µg/day for most pesticides; 18 µg/day for cholinesterase inhibitors), almost all pesticides would be classified safe (mean residue levels for long-term dietary calculations); - Industry has been fighting the non-threshold approach for genotoxic carcinogenic chemicals for decades; adopting TTC will change this long-lasting EU (and global) policy and allow a high exposure of these substances to humans; the threshold approach is not based on science and an untested assumption in regulatory world : - Adopting TTC will mean that the recent accepted "hazard approach" in pesticide Regulation and REACH which bans chemicals with certain properties like genotoxicity, will be completely undermined now genotoxic substances can be classified safe again; - TTC is proposed to be used for impurities/metabolites which industry is obliged to, but reluctant to test. However there can be no doubt the use will be extended to all areas like REACH. Industry lobby club ILSI's top-lobbyist Prof. Boobis (also part of this EFSA panel!) already published industry opinions arguing to extend the use to unknown chemicals in general ; - Adopting TTC will mean the policy of uncertainty factors (10 x 10 calculating vulnerability) is undermined (case of Dieldrin with a factor 30) by the

			unjustified 5th percentile cut-off level. - Adopting TTC will open a 'Pandora's Box' of assumptions of safety and undermine EU policy on many levels like the default drinking water upper level of 0,1 µg/L for pesticides; BASF employees have already published an opinion saying based on TTC it can be watered down to 3 µg/L for metabolites of pesticides; - The acceptable daily intake proposed by TTC exceeds official standards (diazinon, mevinphos and prothiofos) the ADI's (acceptable daily intake) for pesticides; - Moreover it is not in EFSA's remit to work on risk management; this self-tasking job is an unacceptable interference of EFSA with risk management;
220	Conclusions and Recommendations	Cancer prevention & Education Society	There is increasing scientific evidence that chemicals can cause harmful effects at very low levels below the normal dose range used in toxicology studies. Therefore it is important that dose ranges between ng-pg are also studied so as not to exclude substances that cause deleterious effects in these dose ranges. To cut off at higher dosages would be like looking in a building for signs of damp but saying we can"t look in the basement.
221	Conclusions and Recommendations	Kantonales Labor Zürich	We have sent a comment on the draft scientific opinion on Exploring options for providing preliminary advice about possibel human health risks based on the concept of Threshold of Toxicological Concern (TTC)directly to Alan Boobis and Josef Schlatter as it does not fit in this format (text and tables). Our Summary is: The draft opinion on the TTC concept thoroughly deals with the classification of substances and the TTC values. It also carefully checked updates for the basically old data-sets. However it did not adequately validate the concept in describing its usefulness and applicability: the Scientific Committee was requested to "evaluate the relevance and reliability of the TTC approach" and "advise on the application of the TTC concept" (Terms of Reference). The vague description of the application in the "Conclusions and Recommendations" (line 1901ff) is bound to cause difficulties. EFSA should either completely stay out of risk management, which would mean replacing the relevant paragraph by stating who should specify the application of the TTC concept. Alternatively, EFSA should make more specific recommendations on the applications for which it considers the TTC concept adequate. The concept stipulates that the TTC values provide safety in 95 % of the applications. The opinion should interpret this, e.g. by testing the TTC concept for the list of substances evaluated for FCMs. We noticed cases of potentially severely underestimated risk, but also conflicts by the tolerance derived from the TTC concept being far higher than the existing SMLs.

222	Conclusions and Recommendations	RIVM	Section Conclusions and Recommendations: The numbering of the bullets is continued from the summary. It would be more appropriate and logical to restart in this section with bullet numbers a), b), c), and so on. The comments also apply to the corresponding bullets in the Summary. Pg 44 Ln 1901-1905: This paragraph may be read that TTC is only useful for screening or priority setting. Within EFSA context, TTC is already used to conclude on the safety of low level exposures. That goes further than merely screening or priority setting. In addition it could be stated that TTC is a practical way to reduce toxicity data requirements (thus limiting use of animals) for substances with anticipated (or even better: demonstrated) low exposure. Ln 1904: for the sake of clarity it might be helpful to make the line read as: “structure is known but for which there are few……” Ln 1906-1912 This paragraph is superfluous since adequate exposure assessments should be available for every toxicological risk assessment. If the demand is to have conservative exposure estimates to take into account some of the uncertainty included in the TTC approach, it would be more sound and more transparent to reduce the uncertainty in the TTCs or alternatively to increase the assessment factor used to derive the TTCs to take the uncertainty in the TTCs into account. Ln 1923 – 1929 This point is strongly endorsed. See also our comment on Pg 15; Ln 590 (above). pg 45 Lines 1935-1942: Cramer III TTC is partly based on NOELs of OPs and carbamates. Looking at Appendix C, Table 2, quite a number of OPs are present in the lower 10% of the Munro database. It seems logical to recalculate the TTC for Cramer III and propose a new value. It is not clear why the Committee considers it "prudent to maintain the value". For this at least a proper explanatory text is necessary, since it has been extensively studied and argued (section 4.9) that the underlying databases are representative for the “universe of chemicals”. Therefore, if the most toxic ones are singles out to give a separate group-TTC, the remaining group is still representative for the world of chemicals minus this most toxic group. Consequently, the TTC for the remaining less toxic group must be higher than the TTC for the entire “universe”. Ln 1943 – 1946: These lines seem to be contradictory to the derivation of the AchE-inhibitor-specific TTC of 18 ¿g/person/d. Ln 1963 – 1969: It is recognised that structural alerts for genotoxicity would usually “call” for substances or their metabolites, which have direct DNA damaging properties. It might be advisable to include a statement that substances which are demonstrated to be genotoxic through indirect mechanisms (e.g. spindle-poisons causing aneuploidy or ROS-generators) can be evaluated using the TTC approach. Ln 1971 – 1975 This seems to be a rather hypothetical issue, since many of the known structural alerts actually are related to genotoxicity exerted by metabolites (e.g. aromatic amines, secondary amines, PAHs). Of course, in future someone might synthesise a molecule which is not captured by the currently known structural alerts. The same would apply for the currently available testing strategies for genotoxicity (or in fact any kind of toxicity). This bullet is superfluous, and could be deleted and replaced by a general statement in the introduction that the document only reflects the current state of art.

223	Conclusions and Recommendations	RIVM	pg 46 Ln 2006-2012: Bearing our comment on local effects in mind (see pg 30, ln 1274-1278), it might be considered to relate “Portal of Entry effects” specifically to dermal and inhalation exposure, since for oral exposure these are already covered in the TTCs. For the same reason the “substances likely to exert local effects” mentioned in the exclusion categories under figure 2 should be reworded. Pg 47 The contrast between the text and the background in the oval balloons is not high enough. Therefore the text in these balloons is barely visible in black and white printing. Ln 2050 – 2051: These lines are too important to be at the end of the list. They would fit nicely in our comment to pg44 ln 1901-1905. Section recommendations for future work Pg 48 Ln 2078 – 2084: From a toxicological point this may be correct, but for evaluation of combined exposure to substance with the same mode of action (assuming that this is known for data-poor substances) this is problematic. It is nonsense to add up exposures to individual substances on a mg/kg bw/d basis. Only adding up of molar equivalents or toxicity equivalents would make sense. For assessing combined exposure, mol-based TTC as mentioned under 2075-2077 would much more correct if not essential in absence of other data. The issue of aggregated exposure to one substance via different routes might better be discussed in a separate bullet. Again, this would make sense, but as indicated for the same reasons that route-to-route extrapolation is difficult, route-to-route addition is equally problematic.
224	Conclusions and Recommendations	France Nature Environnement	We fully agree with PAN (Pesticides Action network) and are quite worried by the fact that low doses can be harmful, that mixtures can be dangerous .... and that weak populations (children, pregnant women ...) do not seem to be of interest to those who support the project.