EUROPEAN COMMISSION
DIRECTORATE-GENERAL HEALTH AND CONSUMER PROTECTION
Directorate C - Scientific Opinions
Unit C2 – Management of Scientific Committees; scientific co-operation and networks
Scientific Committee on Toxicity, Ecotoxicity and the Environment

Brussels, C2/JCD/csteeop/**TGD-HH25012002**/D(02)

SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE)

Opinion on the

"Revision of the 1996 Technical Guidance Document (TGD) in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) 1488/94 on risk assessment for existing substances [also being extended to provide guidance on risk assessment for biocides under 98/8/EC (excluding human exposure evaluation)]"

submitted for CSTEE opinion on 23 August 2001

Draft revision version on

PART 4/B – HUMAN HEALTH EFFECTS ASSESSMENT

CSTEE opinion expressed by written procedure on 25 January 2002

1. General Comments

The general description of the human health effects chapters is based on a structure that presents how regulators may use and evaluate data. The CSTEE would in addition recommend that a description of requirements for generation of data is included. In many instances, this could simply be a reference to the existing guidelines. However, the chapter on toxicokinetics (3.5) will need a major revision.

Further steps in risk assessment, namely hazard characterisation and risk characterisation are not sufficiently developed and must be described in much more detail in subsequent revisions.

The TGD rightly points to the need for improvements in assessing developmental neurotoxicity, since the foetal and neonatal periods may represent a unique window of sensitivity. The CSTEE also recommends to give more attention to developmental immunotoxicity.

Given that most human exposures are multiple, and not to single chemical entities, the CSTEE suggests that a chapter on combination toxicology be included. In this the general principles of mixture toxicology should be presented where the three basic concepts of joint action of chemicals (simple similar action, simple dissimilar action, interaction) are described. In particular, the possibility for enhancement (adjuvant action) of sensitisation should be mentioned.

The CSTEE also recommends to include a short presentation on the developments, possibilities and limitations for applying genomics and proteomics methods. By use of such methods, hazard identification will certainly be improved in the future. Also, these methods will be instrumental in mechanistic studies.

2. Introduction (3.1)

3.1 Introduction, first two paragraphs
It is proposed that one should use the definitions of the Scientific Steering Committee (*i.e.* Hazard identification, Hazard characterisation and Dose-response assessment) in order to achieve harmonisation. In the second paragraph it should be stated that one should open up to the possibility of using the benchmark dose concept in addition to NOAEL/LOAL. Somewhere in the Introduction the need for mechanistic studies should be pointed out in order to better evaluate the predictivity of animal data to humans.

3.1 Introduction, second indented paragraph
This should rather be the estimation of the relationship between dose (which is both concentration and time) and response (in terms of adversity of an effect, or incidence of response). A distinction is sometimes made between response and effect as qualitatively different measurements. Effects (*e.g.* liver weight) are graded and measured, whereas responses (*e.g.* tumours, which are either present or not) are quantal and counted. This distinction is necessary in order to determine an appropriate mathematical or statistical model for analysis.

3.1 Introduction, fourth paragraph, third line
It is recommended to use the term "reliability", as it is used later throughout, rather than "quality".

3.2.2.2 Human data (comments and suggested changes are underlined)

Page 5, first paragraph
.....Well-documented "negative" studies of good quality and adequate statistical power....

Page 5, second paragraph
....or mortality and may provide the best date for risk assessment. Observational studies include:..... (second bullet) cohort studies, where groups of "exposed"....

Page 5, third paragraph
.....on the magnitude and specificity of the response (although it must be recognised that presently many effects of environmental agents relate to conditions whose aetiology is multifactoral and entail a low relative risk).....using different study designs (independence of databases under analysis is much more important than differences in study design).

Page 5, fifth paragraph
.....different geographical locations of subgroups of the population (*e.g.* job titles), but cannot.....

Page 5, sixth paragraph
Controlled studies on volunteers (3), such as low exposure toxicokinetic studies involving deliberate exposure of individuals. It must be recognised that there are important ethical issues in conducting such studies. When they are already available, they usually allow an assessment of the exposure levels associated with early effects. Such studies include human patch tests for dermal irritation studies. Results are often limited by a relatively small number of subjects, short duration of exposure and low dose levels resulting in poor sensitivity for detecting effects.

Page 5, last paragraph
The criterion "double-blind study design" would be difficult to defend (and perform) for an environmental contaminant!

Page 6, second paragraph
Case reports (4) describe a particular effect in an individual or groups of individuals who were exposed to a substance. In general, they should be considered as a useful source of hypothesis generation. In the case of very specific associations (*e.g.* fibrous materials and pleural mesotheliomas), they can contribute to risk assessment.

3.2.2.3 In vitro data
It should be stated that useful information may be generated by using hormone receptor assays.

3.3.3.1
The present text is scientifically inaccurate and not in accordance with Annex B of Section 3.5. Replace the text after the first sentence by: "In case data on dermal absorption are

available and/or in case data from dermal absorption studies exist, the available information should be used in the light of Annex B of Section 3.5, including the use of default values of 10 and 100% dermal absorption".

3.3.3.2
The LC50/LD50-values should not be used for comparative uptake assessment via different routes of exposure. Please delete the whole first paragraph, only retaining the physiological approach for the approximation of $NOAEL_{inhal}$.

3.3.3.2, page 9, 3rd line from bottom
Typo (LC50 rather than LD50).

3.3.4
Reference should be made to the assessment factor approach Several studies have examined the relationship between NOAELs from semichronic and chronic studies, and also between subacute and chronic exposure. According to Vermeire *et al* (1999; 2001) the geometric mean of the ratios for subchronic/chronic exposure was approximately 2 with a geometric standard deviation of 3.5. Based on such a proposed distribution, the 95th percentile was calculated to 16. The commonly used assessment factor of 10 would coincide with 90% of the variation. The corresponding mean ratio for subacute/chronic exposure was 5 with a geometric standard deviation of 3.5, giving a 95th percentile of 39.

- *Vermeire T, Stevenson H, Pieters M, Rennen M, Slob W, Hakkert B: Assessment factors for human health risk assessment: A discussion paper. Crit Rev Toxicol 29:439-490, 1999*
- *Vermeire T, Pieters M, Rennen M, Bos P: Probabilistic assessment factors for human health risk assessment. A practical guide. RIVM report 601516005, TNO report V3489, RIVM, 2001*

3.3.4 Duration of exposure, page 10, first paragraph
This is a duplicate of the last paragraph on the previous page.

3.4 Dose-response assessment
In general, the CSTEE finds that this section is superficially described. The CSTEE finds that the description of the utility of the benchmark methodology is too negative. The TGD would be an excellent place to advocate changes in study design so as to make use of this valuable methodology.

3.4, page 10, first paragraph, 2nd to the last line
Suggest to add "and is a surrogate for the true NAEL" after " sensitivity of the test system."

3.4, page 10, third paragraph, second line
Insert "statistically significant" after "…at which no…".

3.4, page 10, last paragraph, two last lines
Suggest to write "Also, the data obtained at one dose (NOAEL) are used rather….".

3.4, Page 10, last paragraph and page 11, first paragraph
Suggest to expand the last part to explain how to use the LOAEL for risk characterisation by including the possible use of the benchmark concept.

3.4, page 11, first paragraph
Could include what determines the precision of the NOAEL, namely 1) dose spacing, 2) number of animals per group and 3) sensitivity of the measurement.

3.4, Dose-response assessment, third paragraph, page 11
This is far less important than the Benchmark concept, and should therefore be placed after the first paragraph on page 12. Also, categorial regression is difficult to use in practice and has had little utility. Can be deleted.

3.4, page 11, next to last paragraph
The CSTEE disagrees that, in practice, these two concepts are incompatible. Much more emphasis will presumably in the future be put on the benchmark concept, one should not try to discourage its use by such a statement. The TGD should really support the benchmark concept as a better alternative than simply using the NOAEL for risk assessment. It is possible to make a good fit with only 3 dose levels (plus 1 control) with discrete data. With continuous data it is usually not enough with 3 dose levels, mostly 4 dose levels (plus 1 control). It is envisioned that in many instances not more than 5 dose groups (including 1 control) will be needed. However, this will necessitate some change of the study design, without increasing the total number of animals.

3.4, page 12, second paragraph
Dermal sensitisation is not used for NOAEL derivation.

3.4, page 12, third paragraph
Much less emphasis should be put on lethality, what is needed is to have an indication of acute toxic, not lethal potency.

3.4, page 12, third paragraph
With respect to biocides, the concept of the acute reference dose should be described.

3.4, page 12, last paragraph, last sentence
Should be rewritten to state that it is currently not possible to identify an elicitation dose, rather than there is no consensus.

3. Toxicokinetics (3.5)

Toxicokinetics is an increasingly important tool for extrapolation from high to low doses, from species to species and for the evaluation of interindividual differences and thus invaluable in the risk assessment process. By describing the benefits of application of toxicokinetics in the risk assessment process, the necessary design of studies and their limitations the TGD will encourage the stakeholders in risk assessment to lay more emphasis on toxicokinetic information for evaluation of the health risks of chemicals.

The draft on toxicokinetics of the revised TGD does not meet this goal. Although the subtitles address all relevant aspects of toxicokinetics, the content of these sections need thorough revision, since they either present general statements, do not cover the topic well or are in certain instances even erroneous. Moreover, necessary terms such as target dose, saturation kinetics or steady state conditions are not addressed. These are indispensable for a proper

description of the toxicokinetic behaviour of a compound in the organism. Also, more attention should be given to toxicokinetics during special life stages such as pregnancy.

Unfortunately no document is available which covers all the necessary aspects of toxicokinetics and its application for risk assessment. One recommendation could therefore be to ask experts in the different areas to write specific parts of the text according to their expertise. The following topic areas could be described: 1) Definition and objectives, studies in man, 2) In vitro approaches, 3) Studies in animals, 4) Use of toxicokinetics in risk assessment and 5) Analytical methods used in toxicokinetics. Such a document could also address aspects of mammalian toxicokinetics which may be used for environmental risk assessment.

In relation to the current text, the CSTEE has a large number of comments that indicate the need for a thorough revision. Examples of such comments are given in the following:

First page
3.5.2 Definitions
Toxicokinetics ... "in dependence of time" should be added. Toxicodynamics: It is correct that the toxicodynamic effect is driven by the concentration at the effects site (target dose). Statements that the effects might be reversed or modified are irrelevant here because the necessary information for risk assessment is the correlation between target dose and effect.

3.5.3
This section requires the statement that toxicokinetic studies are designed to obtain species-, dose-, and route-dependent data on concentration-time courses of the parent compound and its metabolites, e.g. in blood, urine, faeces and exhaled air. From these data toxicokinetic parameters e.g. V_{max}, K_m, elimination clearance and bioavailability can be derived by means of appropriate physiological-toxicokinetic modelling. This information permits extrapolations from high dose to low dose, from route to route and from species to species. In lack of *in vivo* data some of the toxicokinetic parameters can be derived from *in vitro* experiments. Such are V_{max}, K_m, metabolic clearance, skin permeation rate or as surrogate for distribution coefficient the log P_{ow}.

In the first sentence *in vivo* or *in vitro/ex-vivo* is mentioned. In vitro should be deleted because the two following parameters cannot be determined in vitro. These sections do not indicate what actually can be obtained from *in vitro* studies.

Second page
2) "Derived information"
This section focuses on oral absorption and apparently does not consider other routes of exposure. "Rate and extent of presystemic (first pass) and systemic metabolism" is relevant for oral exposure, less for other routes. "Rate and extent of excretion ... in faeces, via exhalation ..." cannot be derived from "primary information".

"Half-life and potential for accumulation". It remains obscure what is meant by "potential". Better delete, because potential describes an inherent qualitative property.

"Enterohepatic circulation which may pose special problems for route to route extrapolation" This should be explained in more detail or be deleted.

The last paragraph “It is helpful ...assessed“ is difficult to understand. Accumulation depends on the rates of uptake and elimination. The kinetics of both processes are concentration-dependent and may become saturated. They may also be affected by toxicodynamic processes such as suicide mechanisms or enzyme-induction. Unless these parameters are known it is hard to predict accumulation from plasma/blood concentration time profiles. The last sentence may be deleted.

Third to fourth pages
2) *In vitro* approaches
This sections should provide information on the available *in vitro* test systems that may provide appropriate data to understand kinetics. This may include isolated organs, tissue slices, primary and secondary cell cultures, cell fractions, purified enzymes, reconstituted systems and their ability to provide information on toxicokinetic data, such as quality and quantity of metabolism, data on enzyme kinetics, reactive intermediates and skin penetration rate.

Fourth page
“Animal studies“
This section provides little information on toxicokinetics except the population approach. Generally this approach is helpful to evaluate interindividual differences in human studies, whereas it is of limited relevance in animal studies. In animal studies the interindividual differences are relatively small because the animals usually are of the same strain and the variation of metabolic data within the group of animals studied is small. Precise concentration-time curves determined in single animals are more helpful to understand the toxicokinetics of a compound and are the prerequisite to interpret data from the population approach.

The aspect of non-linearity deserves more elaboration especially to interpret animal data. Non-linear kinetics usually are the consequence of high doses that result in saturation of toxicokinetic processes. Proper evaluation is essential for high dose low dose extrapolation.

Studies in man
Except general statements there is no information on the rationale and design of toxicokinetic studies in man. There is the little exact statement that “The area under the plasma/blood concentration time curve etc. can be used to compare the amount of a chemical in the body in animals and man“. This parameter allows comparing the blood burden, but not the body burden, and not only when the route of exposure is the same.

Fourth to fifth pages
3.5.7 Assessment of the data
1) Analytical Method
In addition to the various parameters listed, the need for a calibration curve, internal or external standards, sample collection and other information to allow evaluation of the data provided, should be included. It remains unclear why validation for the immunoassay is specified, but not those of more commonly applied methods. Criticism on studies measuring total radioactivity is correct, although it should be added that they are helpful for information on the mass balance of a compound.

2) Timing of blood samples
There is no information given on the criteria for optimal timing of blood sampling and some statements need to be amended e.g. the first pass effect cannot be identified from the early time period after administration. AUC should better be replaced by "half-life". It is questionable whether "from a single dose study ..." information on accumulation may be obtained. It should be indicated that the terminal half-life gives better information since usually saturation kinetics prevail shortly after single dose application.

Last paragraph page 4
The statement that bioaccumulation cannot be determined after a single dose administration is not correct.

Sixth to seventh page
3) Experimental conditions for *in vitro* systems
Here at least some criteria should be given on the design and capabilities of *in vitro* systems suitable to obtain kinetic data. The paper of Pelkonen et al addresses *in vitro*-prediction of gastrointestinal absorption that is just one route of human exposure.

3.5.8. Use of toxicokinetic data in risk assessment
Specific aspects needs to be rewritten since various statements are erroneous

Seventh to eight pages
3.6.2 Distribution/accumulation
It remains unclear what the message is. Similar to other chapters the text indicates what usually is made available to the authorities rather than to present the rationale on what data should be made available.

Eighth page
Metabolism
The first sentences are a general introduction to toxicokinetics rather than to metabolism.

Annex A
Gases and vapours
This chapter addresses the uptake of a compound via inhalation. It describes that absorption of a compound depends on the solubility of a compound in the blood. However, the relevance of physical activity on absorption rate has a great impact and must also be addressed (Csanady and Filser: The relevance of physical activity for the kinetics of inhaled gaseous substances. Arch Toxicol 74, 663-672, 2001).

Annex A and Annex B
The annexes present different ranges for log P_{oct} values which favour dermal uptake (Annex A: 1 to 4, Annex B: -1 to 4). The values in Annex B are correct (Annex A should be corrected).

Annex C
The tables on physiological parameters are of little value. They are taken from publications by three authors and provide different figures on the same parameters. Unless there is a discussion which of the parameters or what range can be used for specific purposes these figures are not helpful.

4. Acute toxicity (3.6)

3.6.2.1.1
This section contains 2 essential changes in comparison with the previous TGD.

First change
For a notification (>ton/annum) an acute dermal toxicity study will only be required if signs of systemic toxicity during the irritation are seen. this limitation is not acceptable for the following reasons:

- information concerning the dermal toxicity is essential for performing adequate risk characterisation
- the argumentation (data from notified new substances is not convincing)
- furthermore it is legally questionable if it is possible to deviate from the text of Directive 67/548

Second change
For a notification an acute inhalation toxicity study will not be required if toxicity is seen in an acute oral toxicity study and/or irritation is seen in a skin irritation study. This limitation is not acceptable for the following reasons:

- information concerning the inhalation toxicity is essential for performing adequate risk characterisation when there is considerable inhalation exposure
- the argumentation is not convincing: if the substance does not show toxicity in the acute oral study or is not skin irritating, this does not imply that toxic effects will not occur if inhalation exposure occurs. As the airway system (and the sensitivity to substances) is very different compared to the skin or the gastro-intestinal system in animals and humans
- furthermore it is legally questionable if it is possible to deviate from the text of Directive 67/548/EEC

3.6.1.2, second bullet
It is proposed that this should be changed by describing the objective to do away with lethality (*cfr.* deletion as an OECD guideline).

There is a wealth of data on acute effects from using *in vitro* tests. This should be described as a valuable adjunct to in vivo testing. It should also be mentioned that QSAR may be used to predict acute toxicity.

5. Irritation and corrosivity (3.7)

Also here useful information can be gleaned from in vitro test methods.

6. Sensitisation (3.8)

Skin sensitisation is covered well, because of the availability of good test methods and that it is evaluated on a mechanistic understanding. A validated test methods for respiratory sensitisation is lacking, so that evaluation is based on effects. This situation must be improved. An interesting tiered approach has been very recently been presented in a Dutch

thesis (J.H.J. Arts: Respiratory Allergy Induced by Low Molecular Weight Chemicals in Rats. PhD Thesis, Utrecht University, 2001. ISBN 90-393-2879-X). All lymph node assay positive compounds classified for both and respiratory allergy (stage 1). If additional information is required on specific respiratory potential, an IgE test is done in the BN rat (stage 2). If positive in the IgE assay, one should do inhalation challenge after sensitisation in order to confirm any potential to induce respiratory allergy (stage 3).

It is important to realise that responses resulting in a R42-classification also can be caused by a non-allergic phenomena. The underlying mechanism has to differentiate between respiratory sensitisation and other effects. The IgE-test with Brown Norway rats and the inhalation challenge needs to be validated. The local lymph node assay also indicates respiratory sensitisation potential and not only dermal sensitisation.

3.8.1.1
Most food allergens are protein allergens, outside the scope of this discussion. Most problems with food are non-immunological reactions.

3.8.1.4
There are other approaches using modifications of the lymph node assay without using radioactivity, OECD guideline requires *in vivo* use of radioactivity. For instance, in a modified LLNA, *ex vivo in vitro* labelling is used (van Och *et al.*, 2000).

7. Repeated dose toxicity (3.9)

3.8, page 1
The text "Modified test requirements for closed system intermediates are currently under discussion but not yet agreed".

Add the following text/
Reduced notifications (VIIb)
It is not possible to draw conclusions on repeated dose-toxicity at this notification level. However a specific administration route may be preferred for the repeated dose-toxicity study to be supplied at the level of 1 tonnes/annum. Conclusion ii) can be drawn to identify the route.

Dermal route
Conclusion ii) is reached if:
- the substance is classified, at least as hazardous in an acute dermal toxicity study, and
- dermal exposure is of importance

Inhalation route
- the substance is classified, at least as hazardous in an acute inhalation toxicity study, and
- inhalation exposure is of importance"

3.9.2.1.2, page 86
The following should be added: "In practice, and especially for priority substances, these minimum requirements are not enough for a proper risk assessment".

3.9.3, first bullet
It is difficult to give preference if toxicokinetic or toxicodynamic information is available. Also, species that may be more similar to man than rodents (*e.g.* primates), will not be used in regular toxicity testing.

3.9.1.1, first paragraph, 4th line
Suggest to delete "for the whole life-span" and keep "for the major part of the life-span", since no guidelines advocate treating animals for life.

3.9.1.2, 2nd paragraph, 3.9.2.1, 2nd paragraph and 3.9.5
Here again the CSTEE thinks one should open up to the possibility of using benchmark doses as an alternative to the NOAEL.

3.9.4 and 3.9.5
Here again, the advantages of the benchmark concept as an alternative to risk characterisation based on NOAELs should be pointed out.

3.9.6.1
Risk assessment is not the only aim of animal experimentation. The last sentence is far too restrictive.

3.9.6.5, page 10, first paragraph
Replace the text "…could include a combination of some of the following:" with "…are one or more of the following:", so that also a single test may be sufficient, rather than a minimum of two.

3.9.6.5, page 10, second paragraph
Rewrite to "...a known toxicant that alters thyroid hormone homeostasis...".

8. Mutagenicity (3.10)

3.10.1.1 Definitions
In addition to DNA adducts, DNA strandbreaks should be included in the definitions.

3.10.1.2 Objectives, page 2
Last sentence
"The aims of testing for genotoxicity.....genotoxic carcinogens or to cause heritable damage in humans". It is good that both genotoxicity in germ cells and somatic cells are mentioned. The aim to assess the potential of substances to be genotoxic carcinogens should also be reflected in the classification of mutagens.

3.10.2.1, Industrial chemicals, page 2
The following text should be added after "…greater the needs for further testing":
"For instance, if a structural alert for genotoxicity exists and human exposure is possible, a mouse lymphoma test or a mammalian cell gene mutation test in combination with an *in vitro* cytogenetic test is required. Furthermore, for substances supplied at lower levels (>10 kg/annum but <100 kg/annum), if a structural alert for genotoxicity exists and exposure is possible, an *in vitro* bacterial gene mutation test is required".

3.10.3, page 3, third bullet
Rewrite to "...the possibility of metabolism not active in the system including those in extrahepatic organs...". Fourth bullet can be converted to a question: “Is the substance reaching the target organ?” Last bullet is self-evident and can be deleted.

3.10.4, page 4, second paragraph, 3 third line
Add "alterations in DNA repair". Page 5, last bullet, 2nd to last line: Add "or metabolic switching" after "....mode of action.."

3.10.5.3
Bacterial tests belong in the base level testing so it should be commented.

3.10.5.5
First sentence too strong, not “are” but “considered to be non-genotoxic”.

Table 1, item 5, right hand column
If systemic availability cannot be estimated....”cannot be ascertained”.

9. Carcinogenicity (3.11)

3.11.1.1, page 1, third paragraph
It should also be mentioned that immunosuppression may induce certain types of neoplasia.

3.11.1.1, first page, 2nd to the last paragraph, first line
Insert "non-genotoxic" between "these" and "modes".

3.11.1.1, first page, last paragraph, last line
The meaning of new sentence relative to NOAEL and genotoxicants must be explained (“there are different ways in which a substance may interact with DNA and for some of these genotoxic modes of action....”). Is this the case where oxidative stress may produce reactive oxygen species binding to DNA, when lesions are rapidly repaired or do not seem to contribute in a significant way to induction of neoplasia (*e.g.* formaldehyde)?

3.11.2.2, page 3, next to last bullet
The use of short and medium term carcinogenicity tests deserved a textual description. The outcome of the ILSI-HESI project may be mentioned, and the problems with false positives and negatives described.

Human data (comments and suggested changes <u>underlined</u>)

Page 3, first paragraph
.....Epidemiological data will not normally be available for new substances but may well be available for existing substances which have been in use for many years. <u>Delete from here to end of paragraph, the sense is not fully consistent with wording in paragraph 3.2.2.2. In addition, comments on clusters are obscure, do they refer to cluster detection or cluster interpretation</u>?

Page 3, second paragraph
.....information on actual exposures in setting where they have been carried out, associated dose-responses.....

3.11.3, page 5, fourth paragraph
It is important that one differentiates between mechanisms being of importance for hazard identification and risk characterisation. Some of the examples are not relevant for humans for qualitative reasons (*e.g.* d-limonene and the kidney, saccharin and the urinary bladder), others for quantitative reasons (melamine and the urinary bladder, sulfamethazine and the thyroid)

3.11.3, page 6, Evidence from other experimental data
Cell transformation and intercellular communication data would usually not be present for chemicals with few data.

Page 4, first paragraph
....dose-response analysis and risk assessment. On some occasions (*e.g.* arsenic, benzene) they have revealed the carcinogenic potential of a substance for which at the time there was no experimental evidence of carcinogenicity.

Page 4, second paragraph
The contribution of epidemiological studies to causal inference should be evaluated using generally accepted causality criteria, such as those of Bradford-Hill as modified by Faustman *et al.* (1997). Particular attention should be given to the exposure data in a study (including clear description of the investigated substance(s), interaction with and/or potential confounding from concurrent exposures)and methods used.....are suitable for such an evaluation and publication bias is unlikely to be operating. When the....

Page 4, third paragraph
....can frequently not be ruled out. Reference should be made to the criteria developed by the International Agency for Research on Cancer for distinguishing between agents which are definitely, probably or possibly carcinogenic to humans.

Page 4, fourth paragraph
Epidemiological data suggesting that the carcinogenicity observed in animals is not relevant for humans may inform on the relative sensitivity of humans as compared to animals. However, such data should be very carefully assessed as far as statistical power and intensity and circumstances of exposure.

Page 4, fifth paragraph
The limited...sensitivity of epidemiological studies....

3.11.4, page 7, third paragraph, last sentence
This needs to be expanded so the reader may understand what type of mechanisms one is here thinking of (*v.s.*, oxidative stress?).

3.11.5.1, page 9, last bullet
This should be changed to “Must be shown to be negligible”.

3.11.5.3, page 11
It is stated that a cat 2 carcinogen/cat 3 mutagen should not be further tested for carcinogenicity. However, in some cases a quantitative risk assessment, e.g. for the workplace, might be needed. Therefore add the following: "However, the risk characterisation can make clear that insight in the quantitative risks of the carcinogenic effect might be needed. In such a case, further toxicity testing might be necessary".

3.11.5.6, page 13, first paragraph
It should be stated that the requirement for a long-term carcinogenicity study in one non-rodent species in the biocides directive is wrong (as has been repeatedly pointed out by the CSTEE!).

10. Reproductive toxicity (3.12)

3.12.12.2, page 2
It is stated that a mutagen/carcinogen cat 1-3 or carcinogen cat 1-2 should not be further tested for reproductive toxicity, However, in some cases, a quantitative risk assessment, e.g. for the workplace might be needed. Therefore add the following: "However, the risk characterisation can make clear that insight in the quantitative risks of the reprotoxic effect might be needed. In such a case, further toxicity testing might be necessary".

3.12.6.4, page 8, third bullet
Replace "..and there are low concerns in relation to exposure" by "..and there are no concerns about potential reproductive toxicity (from SAR), and there are low concerns in relation to exposure".

3.12.6.5, page 11, last full phrase
This should be deleted as the testing strategy already covers this. Also, it is certainly not the intention to waive reprotox testing for many substances, especially it is not the intention to do less for existing substances than for new substances.

3.12.7, Additional considerations, 3.12.7.3 (new) Immunotoxicity
Immunotoxicology has been defined as 'the discipline concerned with the study of the events that can lead to undesired effects as a result of interaction of xenobiotics with the immune system. These undesired events may result as a consequence of: 1) a direct and/or indirect effect of the xenobiotic (and/or its biotransformation product) on the immune system; or, 2) an immunologically-based host response to the compound and/or its metabolite(s), or host antigens modified by the compound or its metabolites. When the immune system acts as a passive target of chemical insults, the result can be a decreased resistance to infection, certain forms of neoplasia, or immune dysregulation/stimulation exacerbating allergy or autoimmunity.

Concern for immunotoxicity has resulted in the updating of OECD TG 407 to include an additional set of immune parameters, i.e. weight of thymus and spleen, as well as histopathology of thymus, draining and distant lymph nodes and mucosa-associated lymphoid tissue (*e.g.* Peyer's patches). Currently under consideration is the incorporation of the assay of antibody response to a T-cell dependent antigen, such as sheep erythrocytes.

Regarding effects on the immune system following developmental exposure, well documented information is available on immunosuppression; in contrast, relatively little information is available on allergy and autoimmunity. Concern for immunotoxic effects resulting from pre- and/or postnatal exposure stems from studies showing that the developing immune system is especially sensitive to toxic insults; some compounds having effects that persist well into adulthood. From a risk assessment point of view, important functional parameters that may be affected include cell-mediated immunity, humoral immunity, and the resulting modulation on the progression of diseases including resistance to infections.

Based on the strong immunotoxic effects following perinatal exposure, and the availability of validated immunotoxicologic parameters as mentioned above, it is recommended that such parameters are incorporated into current reproduction toxicity investigations, for instance in the two-generation study (OECD TG 416). Another option would be the inclusion of such testing in the one-generation study (OECD TG 415). To address all potential windows of susceptibility to immunotoxicity in one test, exposure prior to mating, during pregnancy, during lactation and during juvenile development is required, as is addressed in these guidelines. Since in particular T-cell mediated immune responses seem to be affected by developmental immunotoxicants, it is suggested to also include an assay for cell mediated immunity, such as an assay of delayed-type hypersensitivity responsiveness. Additional immune parameters, such as the modulation of immunologically mediated disease, e.g. infections, allergy, and autoimmunity may be studied on a case-by-case basis.

Appendix IX

Although this appendix gives several concrete examples when a specific route is justified, a more structural presentation of criteria is lacking. At least for the following endpoints/aspects, the consequence for the choice of exposure route and the weight of that endpoint should be given:

- corrosive substance
- skin irritating substance
- skin sensitising substance
- volatile substance (vapour pressure>1x1-2Pa)
- substance is inhalable