

# MANUAL FOR INVESTIGATION OF HPV CHEMICALS

## CHAPTER 3: DATA EVALUATION

### 3.2 Guidance on the Development and Use of Chemical Categories in the HPV Chemicals Programme<sup>1</sup>

#### 3.2.1 Introduction

1. There are approximately 5000 chemical substances on the OECD List of High Production Volume Chemicals (last update 2004). The OECD List of HPV Chemicals serves as the overall priority list from which chemicals are selected for SIDS data gathering and testing and initial hazard assessment. The first step in making an initial assessment of an HPV Chemical is to ensure that there is adequate information on each of the elements which make up the Screening Information Data Set (SIDS). If adequate information is not available then additional data is needed to complete the SIDS for a HPV chemical.

2. For reasons of resources and animal welfare, it is important to limit the number of tests to be conducted, where this is scientifically justifiable. One approach is to consider closely related chemicals as a group, or category, rather than as individual chemicals. In the category approach, not every chemical needs to be tested for every SIDS endpoint. Rather, the overall data for that category must prove adequate to support a screening-level hazard assessment. The overall data set must allow the estimation of the hazard for the untested endpoints.

3. An additional advantage of a category assessment approach is that identification of consistent patterns of effects within a category in itself increases confidence in the reliability of the results for all the individual substances in the category, compared to evaluation of data purely on a substance-by-substance basis.

4. All assessments require regular review and periodic update as new information is generated. Because this is a complex area, and one in which experience is growing, the review and update of category assessments is particularly important. This will help to ensure scientifically acceptable results consistent with the original premise for the category and that methodology associated with category assessments is continually improved.

5. This document has been developed based on existing OECD SIDS cases involving categories, guidance issued under the US HPV Challenge Programme and other US EPA programmes, and on the experience gained from the OECD Workshop on the development and use of chemical categories held in January 2004. The document will be updated as further experience is gained. Furthermore, this document addresses the actual formation of categories for test plan and hazard assessment purposes. It does not address issues of presentation. These are dealt with in section 2.3.5 as well as Annex 2 (supplement 1) of Chapter 2 of this Manual.

#### 3.2.2 Definitions and explanation of the chemical category concept

6. A chemical category is a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. These structural similarities may create a predictable pattern in any or all of the following parameters: physicochemical

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<sup>1</sup> This document was prepared by the OECD Secretariat based on the agreements reached in the OECD Existing Chemicals Programme up to May 2005.

properties, environmental fate and environmental effects, and human health effects. The similarities may be based on the following:

- a common functional group (e.g., aldehyde, epoxide, ester, metal ion, etc.); or
- the likelihood of common precursors and/or breakdown products, via physical or biological processes, which result in structurally similar chemicals (e.g., the “metabolic pathway approach” of examining related chemicals such as acid/ester/salt); and,
- an incremental and constant change across the category (e.g. a chain-length category).

Different types of categories are described in more details in section 3.2.5.

7. The applicability domain of a chemical category identifies the physicochemical property space within which the chemical category is considered to be reliable. The applicability domain is a concept borrowed from the QSAR field<sup>2</sup>. In the context of a chemical category, it can be considered to identify the ranges of physicochemical, environmental, toxicological and/or ecotoxicological properties within which reliable estimations can be made of missing data points, by the use of trend analysis (interpolations and/or extrapolations), read-across, structure-activity relationships (SAR), quantitative structure-activity relationships (QSAR), activity-activity relationships (AAR)<sup>3</sup> (see Annex 2 for further definitions). It can also be considered as a set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members. To illustrate the concept of applicability domain, it might be observed that the category of ethylene glycols show trends in certain properties in proportion to the chain length of the glycols, but that these trends are only applicable within a *defined* range of chain lengths.

8. A chemical category can be represented graphically as a two-dimensional matrix in which different category members occupy different columns, and the different category endpoints occupy different rows (Figure 1). Data gaps can be filled in by one or more of the following procedures: qualitative read-across, quantitative read-across, use of SARs, use of QSARs<sup>4</sup>.

9. Read-across can be regarded as using data available for some members of a category to estimate values (qualitatively or quantitatively) for category members for which no such data exist.

Qualitative read-across can be regarded as the application of SAR by using data that are internal to the chemical category. The process involves: a) the identification of a chemical substructure that is common to two or more members of the category (which are therefore analogues); and b) the assumption that the presence (or absence) of a property/activity for a member can be inferred from the presence (or absence) of the same property/activity for an analogous member. This assumption implies that analogues behave qualitatively similarly, and is usually the result of an expert judgement evaluation rather than a more formal (mathematical) analysis.

Quantitative read-across involves the identification of a chemical substructure that is common to two or more members of the category (which are therefore analogues), and the assumption that

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<sup>2</sup> The analogy between (Q)SARs and chemical categories is made deliberately throughout this document. A chemical category can often be seen as a set of QSARs on a small scale for the different endpoints, with the advantage that all the underlying data are transparently available to the assessor. For larger categories it is possible that several different relationships can be established for a single endpoint and different members of the category (e.g. through trend-breaks) thereby defining “subcategories”.

<sup>3</sup> The experience with Activity Activity Relationships (Q)AARs is currently limited and therefore this approach is not routinely used within the OECD HPV Chemicals Programme. The concept is presented in this document for completeness sake. Further experience with this concept will lead to revisions of this document.

<sup>4</sup> together with consideration of those physico-chemical properties that determine uptake from the different environmental compartments in the case of ecotoxicity endpoints

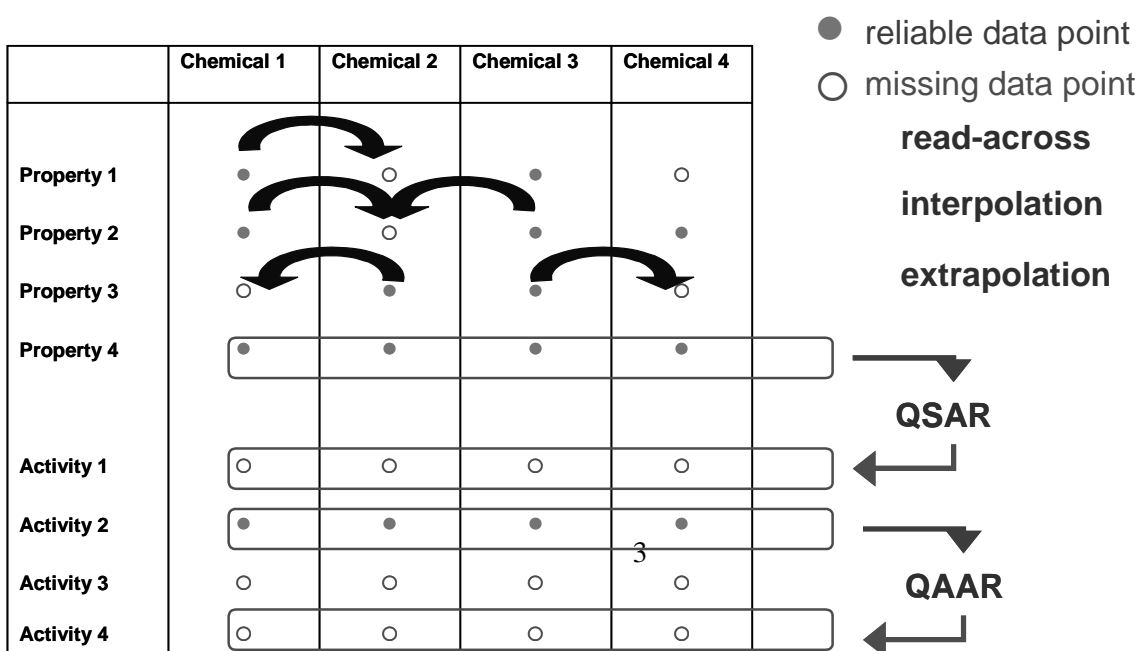
the *known* value of a property for one member can be used to estimate the *unknown* value of the same property for another member. This assumption implies that the potency of an effect shared by different analogous chemicals is similar, and is usually the result of an expert judgement evaluation as well as a more formal (mathematical) analysis.

10. Data that are external to the chemical category (data from an analogous surrogate chemical) can also be applied by using SARs. The process involves: a) the identification of a chemical substructure that is shared by a category member and by one or more surrogate chemicals; and b) the prediction of the presence or absence of an effect/activity for a category member on the basis of its similarity to the surrogate chemical. Data from surrogate chemicals should not be used selectively for only those endpoints which support the category, unless justified on a scientific basis. During the analysis to determine if the surrogate chemical is suitable, it may often be necessary to review data for endpoints other than only the endpoint of concern. However, this analysis should be performed by the Sponsor country at the initial Test Plan phase for possible review by other member countries rather than presented in the assessment documents (SIAR, SIAP). Preparing a comprehensive dossier for the surrogate chemical with Robust Study Summaries for the final SIDS documents would not be necessary.

11. QSARs can be applied by using data that are internal and/or external to the chemical category. A QSAR is a model that makes predictions of an activity (or property and in some cases the potency of the activity) from a numerical measure of chemical structure (or physicochemical property) [see also section 3.3].

12. Trend analysis can be applied when the members of a category exhibit a series of increasing or decreasing values for a given endpoint. Interpolation is the estimation of a value for a member using measured values from other members on “both sides” of that member within the defined category spectrum (see Figure 1), whereas extrapolation refers to the estimation of a value for a member that is near or at the category boundary using measured values from internal category members (see Figure 1). In general, interpolation between category members is preferred to extrapolation. Especially for larger categories there may be breaks in trends, affecting the reliability of extrapolation. However, in certain cases, such as where toxicity does not change among tested category members, extrapolation to other category members may be acceptable. Interpolation can be performed with a certain confidence when the series of values is monotonic (all increasing or decreasing), but guidance and caution is needed in the case that one or more values are outliers to the trend.

**Figure 1 Graphical representation of a chemical category and ways of filling in data gaps**



13. Within a category different members can be selected to demonstrate the pattern or trend of interest - i.e., those selected for a category approach for environmental effects endpoints may not be suitable for assessing human health effect endpoints. Furthermore, within a category, correlations might be established for different members of the same category depending on the property (thereby establishing “sub-categories”). For example, for categories constituted of chemicals with increasing chain length, a trend might be seen for aquatic toxicity for the lower chain chemicals while a cut-off in toxicity is seen starting with a given chain length. On the other hand a correlation might be seen for another property (e.g. acute mammalian toxicity) over the whole category.

### 3.2.3 General Approach for Developing Categories

14. Categories accomplish the goal of the HPV Chemicals Programme - to obtain screening level hazard information - through the strategic application of testing to the category. If these test results show that the chemicals in the category behave in a similar or predictable manner, then the relational features described in figure 1 can be used to assess the chemicals in lieu of conducting additional screening-level testing.

15. Developing chemical categories can be considered a stepwise process (see Figure 2 for a schematic of the process and Annex 1 for examples).

- **Step 1: Identify proposed category and its members**

A category can be defined in a variety of ways. Traditionally, as outlined on section 3.2.2, category definitions have referred to chemical classes with a common functional group (e.g. epoxides) or chemicals with an incremental and constant change across the category (e.g. a chain-length category). Some categories have been defined in terms of a metabolic pathway i.e. they have a stepwise metabolic pathway producing the different members within the category with each step.

A category definition should describe the molecular structure a chemical must have to be included in the category, including criteria such as carbon chain length, functionality, chemical or metabolite equivalence considerations, etc., and should list the specific substances covered.

The category should also be described (characterised) in terms of:

- a) The relational features of the category, i.e. the chemical similarities (analogies) and trends in properties and/or activities that collectively generate an association between the members. The relational features can be regarded as the “connective tissue” that hold the category members together. Relational features include SARs, QSARs, AARs, examples of read-across, and examples of trend analysis (interpolations and extrapolations).
- b) The applicability domain of the category, i.e. a set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members (see paragraph 7).

Whilst the selection of a particular chemical category will normally be guided by the presence of a number of HPV chemicals in the category, it should be noted that a category may also

contain other substances that are not HPV chemicals (or indeed, are not necessarily commercially available). These chemicals are legitimate candidates for the category, and may in some cases prove to be relevant candidates for further testing in order to evaluate the properties of the category as a whole<sup>5</sup>.

In identifying a category, it is important that all potential category members are described as comprehensively as possible.

For potential members of a category, all relevant CAS numbers should be selected. For some substances, there may be more than one CAS number, and studies may contain relevant data reported under different CAS numbers. Due to historic reporting errors, a CAS number used to describe a substance may not accurately describe the substance as marketed. The CAS numbers of members of the category should also be checked against different inventories (e.g. TSCA, EINECS, ELINCS, Customs Inventories etc.) as these inventories can provide an indication as to which CAS numbers are used for marketing the substances and hence for which CAS numbers additional data might be available.

It is important that information on the purity and impurity profiles of all potential category members is collected at the same time as details of the molecular structure. Differing purity or impurities could influence the overall toxicity. For example, a category member may contain a particularly toxic impurity that is not present in the other substances making it difficult or impossible to draw conclusions on the toxicity of other substances in the category. It is therefore important that category members have similar purity profiles or, where they differ, the effect of the differing purity profiles is known.

- **Step 2: Gather published and unpublished data for each category member.**  
Gather published and unpublished data on physicochemical properties, environmental fate and effects, and health effects for each member of the category. This should include all existing relevant data and not be limited to the SIDS endpoints (e.g., metabolism and cancer studies are relevant but not part of SIDS). Prepare the SIDS Dossiers for each individual category member. Specific guidance on how to prepare SIDS Dossiers for chemical categories with the IUCLID software can be found in Chapter 2, Annex 2, Supplement 1.
- **Step 3: Evaluate available data for adequacy.**  
Evaluate available data for adequacy using the OECD Guidance for Determining the Quality of Data for the SIDS Dossier (see section 3.1).
- **Step 4: Construct a matrix of data availability.**  
Construct a matrix of data availability (SIDS endpoints vs. category members) arranged in molecular weight order (or some other fashion indicating the structural progression of the category). Indicate in the cells of the matrix whether data are available or unavailable, as well as the available key study results.
- **Step 5: Perform an internal assessment of the category**

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<sup>5</sup> It is recognised that in many cases the formation of a category is also dependant on which chemicals are manufactured by the consortium of companies sponsoring the category. While these considerations can legitimately influence the formation of a category, they are independent of the scientific analysis of a category and therefore not further addressed in this guidance document.

In this step, an internal assessment of the category is performed. The internal assessment consists of:

- a) identification of the relational features that collectively generate the association between the category members. These relational features are proposed on the basis of *existing data*, which may be internal and/or external to the category.
- b) use of the relational features to fill data gaps (empty cells in the category matrix) or fill in matrix cells containing data of uncertain quality.

In this context, the term “internal” is borrowed from the QSAR field, in which the internal assessment of a QSAR model refers to an assessment of the model performance by using the same data that were used to develop the model.

Evaluate the category approach to determine whether there is a correlation among category members and each SIDS endpoint by looking for patterns in the matrix. The same category members do not have to be used for each evaluation, i.e., the members selected for environmental fate may be different from those used to evaluate toxicology effects.

- If there are adequate data for a given SIDS endpoint, but no apparent pattern, the proposed category may not be appropriate and so testing may be required for all remaining category members for that SIDS endpoint. However, an alternative category proposal may be developed e.g. the analysis might suggest that the category should be divided. (go back to Step 1).
- If there are adequate data that correlate well, the category may be appropriate and a category test plan proposal should be prepared (Step 6).
- If adequate data do not exist, but the structure-based category is reliable for one or more SIDS endpoints, then a category approach may still be proposed (go to Step 6).

When establishing trends in data, laboratory and experimental variations should be considered. Similar species/strains, endpoints and test protocols should be compared. Deviations from a trend should be clearly identified and possible reasons for the deviations laid out in the category analysis.

The category approach is most robust when a quantitative trend between the category members can be established. A lack of observed toxic effects for a chemical substance in a study of a specific end-point (especially if no dose-relationship can be established because no effects are observed at the highest dose tested) is usually of limited value to establish the robustness of the category.

- **Step 6: Prepare category test plan.**

Category test plans (Step 6 of Figure) should include a category definition, rationale, and matrix of data availability (see example category test plans in Annex 1.) and be accompanied by SIDS Dossiers for each category member.

The rationale supporting a category definition should be as simple and transparent as possible, and should explain why the existing data and proposed testing data allow interpolation or extrapolation to other members of the category that have no data or proposed testing.

The test plan needs to summarise the adequacy of the existing data, and how the proposed testing will adequately characterise the category.

The matrix of data is an essential part of the test plan and provides a useful tool for consideration and presentation of the available data (see Annex 1). Assuming the SIDS endpoints are rows in the matrix, each row must have data in at least one cell. If toxicity is expected to vary in a regular pattern from one end of the range of category members to the other end (e.g. high toxicity to low toxicity), samples chosen for testing should bracket both ends of toxicity. If the category is large, testing also needs to be performed and/or data should be available for one or more members in the middle of the range of toxicity. Any change in a tendency for a property should be accompanied by data in the adjacent cells in order to define the limits for the resulting subsets of the category or sub-categories. Assuming the columns are the category members, one or more columns may have all empty cells, i.e. no test data available. There are no rules for the number of columns and cells that must be filled nor the number that can be empty. Acceptability of the matrix will depend on the number of members in the category, the SIDS endpoint, and the confidence in the interpolation and extrapolation.

When selecting a sample to test, it should be representative of the substance marketed, including the presence of any manufacturing impurities (see section 2.3.3).

It should be noted that the category test plan is intended to provide information about the properties of the group as a whole rather than the properties of any specific, individual compound. This approach is very different from the approach widely used in the current evaluation of both new and existing chemicals, where the test plan is focussed on obtaining data on an individual compound of commercial interest. A category test plan may thus identify as key substances for testing substances of little or no commercial importance. Whilst in some cases this may even require the synthesis of chemicals specifically for this purpose, the approach may still prove more economical, both in terms of expense and numbers of animals used for testing, than a more conventional testing strategy based on individual commercially available chemicals.

At this point in the process, the sponsor country may consider to submit the test plan to the other OECD member countries for consultation.

- **Step 7: Conduct the necessary testing.**
- **Step 8: Perform an external assessment of the category and fill data gaps**

In this step, some or all of the relational features are assessed by checking whether the predictions they make for data gaps (or data points of dubious quality) are accurate on the basis of *newly-generated* experimental data, obtained in Step 7.

In this context, the term “external” is being borrowed from the QSAR field, in which the external assessment of a model refers to an assessment of the model performance by using independent data different from that used to develop the model.

Add the new data to the SIDS Dossier for the relevant category member and evaluate whether the existing data and the new data support the proposed category.

- If the results support the category, the testing phase is complete. A SIAR for the category of chemicals should then be prepared including a category analysis. The category analysis will include a summary of the one or more SIDS endpoints in which the category “holds”, including the interpolation/extrapolation of test results to the remaining, untested matrix cells (see below). The SIAR would receive Member country review at the SIAM meeting.
- If the results do not support the category return to Step 5. Further testing may be carried out, members of the category may be changed (e.g. dividing the category as appropriate), or the category proposal may be dropped altogether. The latter implies that testing will then be done to fill all appropriate SIDS endpoints for each HPV category member.

As indicated in section 3.2.2, data gaps are filled by read-across, extrapolation or interpolation. This is specific to each category. No definitive guidance can be provided for the moment. A few examples are provided in Annex 1.

Available options for filling data gaps include:

1. Qualitative: it is concluded that all the members of the (sub)category do or do not possess a particular property e.g. *in-vitro* mutagenicity.
2. Quantitative: it is concluded that all the members of the (sub)category possess a particular property with a similar potency or evolving according to a regular pattern. Data gaps can be filled e.g.
  - by using the value from the closest analogue in the (sub)category;
  - by using a worst case approach i.e. using the value from the most hazardous substance in the (sub)category, or in case of interpolation, the value from the most hazardous of the two closest analogues (see figure 1);
  - by estimating quantitatively the potency of the property from the potency of the two closest analogues or from the regular evolution of this potency over the different (sub)category members.

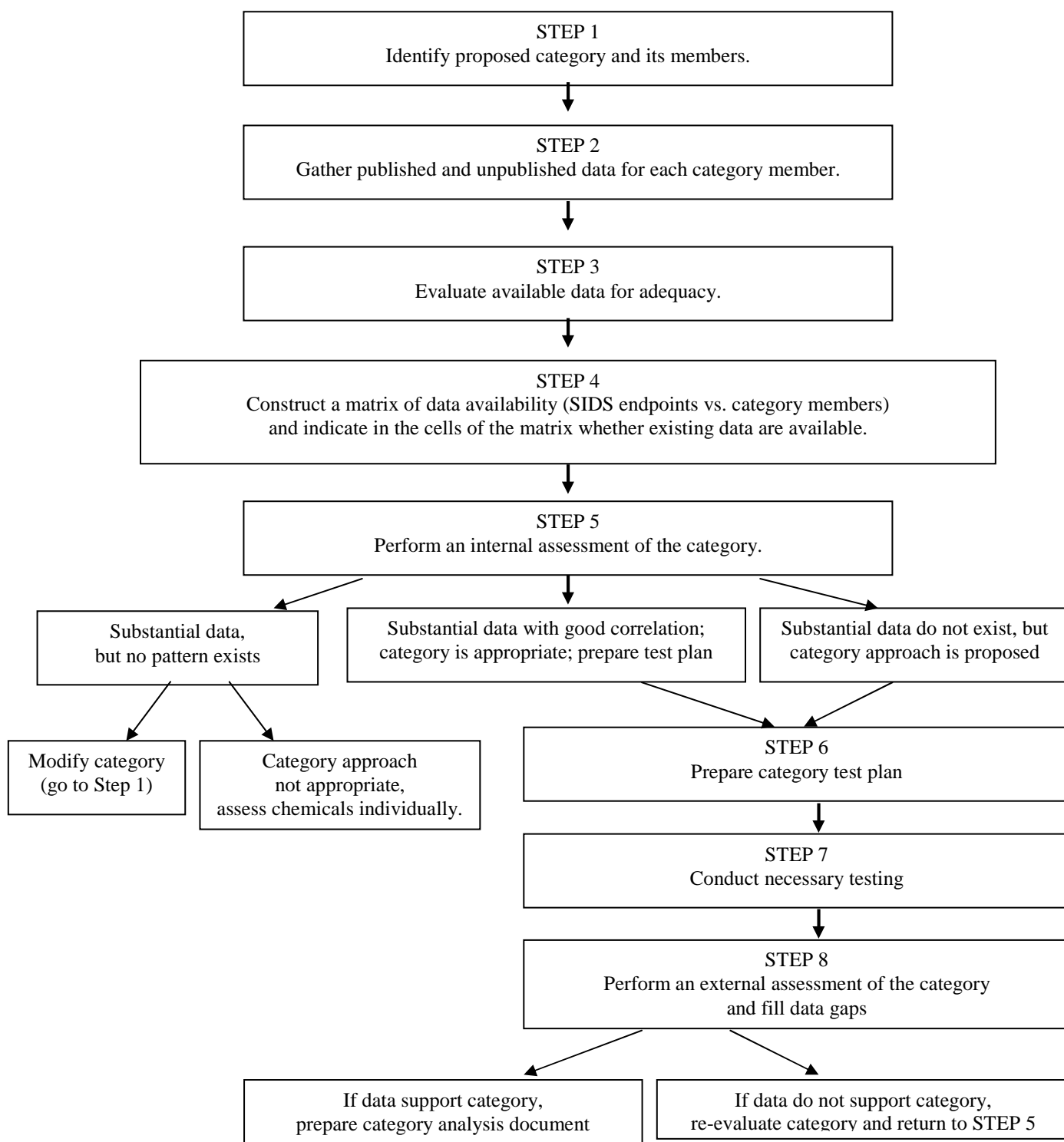
There is currently only limited experience with quantitative data gap filling for toxicological endpoints. It should be applied with caution and the guidance will be revised as soon as more experience is available.

QSARs could be used to support proposals for filling data gaps by any of the mechanisms described above.

For categories composed of complex substances, approaches like the toxic equivalency factors or toxic units approach could be investigated to fill data gaps.

The mechanism by which the data gaps are filled in a given category should be any case be described transparently in the SIAR.

**FIGURE 2: PROPOSED PROCESS FOR DEVELOPING CHEMICAL CATEGORIES**



### 3.2.4 Use of QSARs for the Development of a Category

16. Greater confidence and further demonstration of the category approach may be gained through applying QSAR models on all category members for a given endpoint in case reliable QSAR models are available for the category members and the endpoint. QSARs can contribute at all stages of category development and consideration. Based on experienced assessment of the quality of output taking into account limitations and strengths of a range of models, QSARs may contribute not only for endpoints and compounds within categories where there are no relevant data but also in the interpretation of weight of evidence for mixed datasets and analysis of trends.

17. The output of QSAR modelling is particularly valuable in hypothesis generation and testing for step 1, “identifying the structure-based category and its members”. Analysis of physico-chemical and (eco)toxicological data from the members of a category should demonstrate clear relationships between those members; outliers can then be investigated for their ‘eligibility’ for membership. It also permits hypothesis testing of several possible combinations and permutations for category definition. The more transparent, evolving analytical models provide access to detailed description of relevant data in the training sets. This can facilitate initial consideration of trends to establish the nature and bounds of the category.

18. QSAR modelling can also assist at this stage in defining the appropriate bounds of the proposed category, through consideration of measures of similarity for chemical descriptors in the models. In the more transparent, evolving analytical models, the bounds of this similarity can be specified and the category defined accordingly. In some cases, this can lead to the definition of a more extensive category than was originally envisaged.

19. In addition, QSAR can in some cases be used to assess similarities in metabolic pathways across the group, and this information can be helpful in assessing similarities and differences within the category.

20. Results of QSAR modelling are also relevant to step 2 (“Gathering published and unpublished data for each category member”). In addition to contributing to trends analysis for potential members of the category where no data have been identified, considered output of a battery of models can also add weight of evidence to increase confidence in trends analysis, where the pattern is not clear or consistent based on available data. For example, evaluated QSAR output may contribute where dose spacing or non-comparability of experimental protocols in available studies for different members of the category precludes meaningful analysis of quantitative trends of effect levels. In compiling this information, however, it is important to distinguish where the models contribute additionally to identified experimental data – i.e., that they are not simply duplicating the information, based on replication of its inclusion in their training set. The ease with which this information can be accessed for various models (if at all) varies, depending upon degree of transparency.

21. In relation to step 3 (“Evaluate available data for adequacy”), for QSAR modelling, this requires consideration of aspects related to the training sets and the models, themselves. Relevant aspects include criteria for inclusion of and nature of data in the training sets, the nature of the analysis for consideration of similarity, the criteria for weight of evidence for delineation of a positive/negative response and the nature of validation of the models and aspects thereof, including concordance, sensitivity and specificity for specific endpoints and subsets of chemicals. For characterization of hazard for related endpoints, critically evaluated QSAR output can be combined with weighting of the endpoints themselves (e.g., *in vivo* versus *in vitro* genotoxicity) as a basis for meaningful contribution to hazard characterization, particularly where data are lacking or mixed.

22. For step 4 (“Construct a matrix of data availability”), then, it will be important that results of QSAR modelling be clearly distinguished from those which are based on data. As indicated above, only evaluated results of QSAR modelling which contribute additionally to weight of evidence determinations or quantitative trends analysis should be included. This would include, then, only results for modelling, where evaluated output meaningfully contributes to weight of evidence or trend analysis (this could be for substances where there are no data or where datasets for category definition are uninformative or mixed).

23. For step 5 (“Perform an internal assessment of the category”), the output of QSAR modelling introduced and considered as outlined above can contribute to trend analysis for compounds in the series both for those for which there are data and those for which there are not. Through measures of similarity, it can also contribute to delineation of the bounds of the category.

24. For step 6 (“Prepare category test plan”), where critically evaluated output of QSAR contributes meaningfully to trend analysis, it may obviate the need for testing of certain members of the category. Rationales need be based on well documented critical evaluation of the output of batteries of models, with clear delineation of strengths and limitations and take into account availability for other members of the category and consistency overall of critically evaluated QSAR output and data.

25. For step 8 (“Perform an external assessment of the category and fill data gaps”), the principles outlined above for consideration of QSAR in development of the test plan are also relevant in considering their contribution to the initial assessment. This contribution must necessarily be based on critical evaluation of the output of a suite of models, based on an understanding of their relative limitations and strengths for the specified application.

### **3.2.5 Guidance on different types of categories**

#### Chain length

26. These are defined as categories showing an incremental, and usually constant, increase in chain length across the category. There is an assumption that each category member exhibits the same toxic mode of action. Examples are the homologous series of alpha-olefins (see Example A in Annex 1) where each category member differs by a  $-CH_2-$  unit and the ethylene glycols where there is an incremental increase in the number of  $CH_2CH_2O$  groups.

27. Categories defined by chain length generally show an incremental change in molecular weight and other physico-chemical properties such as water solubility or Log Kow. However, not all properties will necessarily exhibit a linear relationship with chain length and care must be taken in making assumptions about such trends. For example, the alpha-olefins show declining acute toxicity to fish with increasing chain length and decreasing water solubility. There is an apparent ‘cut-off’ point between the C8 and C10 chain length at which acute toxicity to fish is no longer observed due to the decreasing water solubility. For aquatic toxicity, the interplay between decreasing water solubility and increasing log Kow – a key indicator of uptake from water - with increasing carbon chain length is often important in determining this cut-off point. Similarly, a trend of increasing molecular weight may lead to decreasing systemic toxicity as absorption decreases and there may be a change of physical state of the category members as chain length increases.

28. Careful thought should be given to selecting the boundaries of a chain length category. The cut-off points described above may provide useful boundaries. The potential scope and size of a chain length category may be larger than that covered by a particular manufacturer or consortium. Where possible, well-characterised substances which are not HPV but which fit into the series should be included. There

may be cases when testing the end members of a chain length category is not appropriate. For example where the existing data indicates that the cut-off for toxicity occurs earlier in the series it may not be necessary to test the end member for that endpoint.

29. QSARs can be used to help justify the category and fill data gaps. In general, substances at either end of a chain length category should have all SIDS endpoints fulfilled, preferably with test data. This permits interpolation of data to the other category members rather than extrapolation and increases confidence in the read-across. For example, a linear regression has been used to predict acute aquatic toxicity of long chain alcohols. For categories where there is more than one variable, such as variation in chain length and degree of branching of the chains, more category members are likely to be required to bring confidence to the interpolations being made.

### Metabolic pathways

30. The underlying hypothesis for a metabolic series is a sequential metabolism of a parent chemical to downstream blood metabolites that are chemicals of interest. Hazard identification studies with the parent compound could then be used to identify the hazards associated with systemic blood levels of the downstream primary and secondary metabolites and once quantified, can be used in place of studies using direct exposure to primary and secondary metabolites themselves. In certain instances, the metabolism of the parent compound within barrier tissue (e.g. lung or gut tissue) occurs so rapidly that the initial primary metabolite is the predominant chemical found within the blood. Under these circumstances data from hazard identification studies conducted with that primary metabolite itself can be used to identify hazards for the parent compound. PBPK or PBPD models may help to define categories. The metabolic pathway approach is usually reserved to some toxicological endpoints. For physico-chemical properties, environmental fate and ecotoxicity, information on the parent compound would need to be available.

31. The first technical issues faced when forming a metabolic series is to determine if the metabolism that is assumed to occur does occur. This is necessary before moving any further in developing a metabolic category and preferentially should be determined *in vivo*. In certain instances, *in vitro* metabolic studies can be used to help identify metabolic pathways, but the definitive evidence should be conducted in whole animals. The primary and secondary metabolites should be detected either in the blood or tissue. Primary and secondary metabolites that cannot be readily determined in blood or tissue should not be candidates for a metabolic series approach without some limitation placed upon the use of the information.

32. The second technical issue pertains to the level of evidence required to describe the metabolic processes. Direct measurement of the parent chemical and primary and secondary metabolites in the blood in an *in vivo* exposure is the recommended standard. The level of evidence required to presume that there will be blood-borne levels of primary and secondary metabolites following exposure to parent chemical, will have to be determined on a case by case basis. Certain metabolic processes are ubiquitous and well understood and these can be presumed to occur without performing *in vivo* experiments in every instance. Other metabolic processes are not part of normal metabolism or require enzyme induction. These metabolic processes may not be well characterized and should not be assumed without specific *in vivo* evidence of blood levels of primary and secondary metabolites.

33. The third technical issue provides a limitation for the metabolic approach to forming categories. The metabolic category reasoning is only useful for identifying hazards related to systemic blood levels of the parent compound and/or primary and secondary metabolites. Other endpoints of hazard identification studies that are dependent upon site of contact effects (e.g. eye, skin, respiratory tract irritation, irritation to gastric mucosa) cannot be addressed using the metabolic category logic. These sites of contact effects are often due to the physical chemical property of the chemical in question and therefore may differ considerably between the parent compound and primary and secondary metabolites. In addition, tests that

identify unique structural characteristics (e.g. skin or respiratory sensitization) or are dependant upon physical chemical properties (e.g. volatility and LC50 values) should not be considered as part of metabolic category because these properties may not be similar amongst the various members of the metabolic series.

34. An additional limitation of the metabolic categories approach is that metabolism and toxicokinetics experiments have to be conducted with the parent compound. Typically, these types of studies are not SIDS elements and therefore would require a sponsor of the chemical to do additional work beyond what is normally considered necessary. However, it should be recognized that the savings involved (numbers of animals used, testing costs) could be considerable compared with generating data for each metabolic category member for each endpoint of systemic toxicity. Since the OECD HPV Chemicals Programme is a screening level program that is interested in identifying hazards related to systemic blood levels, it should not become necessary to provide definitive toxicokinetic evidence or develop a toxicokinetic model for acceptance of hazard identification studies as relevant for the primary and secondary metabolites.

35. An additional advantage of using the metabolic category toxicity data is that in certain instances, higher systemic blood levels of a chemical can be achieved from metabolic pathways than if the primary or secondary metabolite was administered directly. For example, if a material is corrosive or has limited volatility, higher blood levels may be found following the administration of the parent compound than if the primary or secondary metabolite was administered directly to the animal.

36. The following specific issues should be taken into account when developing a metabolic pathway category, according to the stepwise procedure described in section 3.2.3

- *ad* step 1: Provide definitive information on the metabolism of the parent chemical to the primary and secondary metabolite. This information should also include, preferably, a time course data for either blood or tissue for both the parent chemical as well as the primary and secondary metabolites.
- *ad* step 2: The metabolism experiment should be examined to determine, if in fact, the primary and secondary metabolites are formed, if they achieve appreciable levels within the blood and/or tissues and determine basic toxicokinetic parameters for the parent material. For example, the  $T_{1/2}$  for elimination for the parent chemical should be determined if possible. If the metabolism of the parent chemical to the primary metabolite is rapid and is thought to occur within barrier tissues, then it may be appropriate to use hazard identification studies from the primary metabolite to identify hazards associated with exposure to the parent chemical.
- *ad* step 3: If there are appropriate hazard identification studies that have been conducted with the parent chemical or primary or secondary metabolites for similar toxicity endpoints, then these studies should be examined to see if these materials have similar toxicity. If data is not available for the metabolic series in question and a study is to be designed and conducted, then the parent compound should be tested, so that blood levels of all category members will be present. The toxicokinetic and metabolic experiments that provide the basis for the metabolic category should have robust summaries prepared and be included in the SIDS Dossier for the parent chemical, primary and secondary metabolites. Within these robust summaries a table should be included detailing the relative blood levels of the parent chemical, primary and secondary metabolites.

- *ad* step 5: A quantitative analysis between exposures of the parent chemical and the primary and secondary metabolite is not necessary as the point of the OECD HPV Chemicals Programme is to provide hazard identification studies for these materials, not a quantitative analysis as would be done for risk assessment purposes. If the chemical becomes a chemical of concern, then additional toxicokinetic analysis (including preparing a model) may be appropriate, but for the purposes of the screening level OECD HPV Chemicals Programme it is not necessary.

37. The metabolic approach should not be used for environmental toxicity endpoints unless the metabolism of the parent compound to the primary or secondary metabolite can be demonstrated within the test species in question. Whereas it may be appropriate to extrapolate within mammals, it may not be appropriate to extrapolate between amphibia and fish or insects and other species due to the difference in the metabolic processes and enzymes present within those species.

38. On the other hand the same concept underlying the metabolic pathways can be used for environmental degradation processes. For example, for a substance which hydrolyses very rapidly in aquatic test systems (half-life < 1 hour), the aquatic toxicity endpoints can be covered by the test results with the degradation product(s) [see also the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures No. 23, ENV/JM/MONO(2000)6].

#### Chemical mixtures

39. Categories can sometimes apply to series of chemical reaction products or chemical mixtures<sup>6</sup> that are, again, related in some regular fashion. Analogous to the basic “discrete chemical” category model, in a mixture category some, but not all, of the individual mixtures may undergo testing. Annex 1 illustrates this using a category made up of linear alkylbenzene mixtures. This example was used to assess these chemicals in the OECD HPV Chemicals Programme. This is a relatively simple example of the type of approach that can work in the HPV Chemicals Programme. Additional guidance for other types of mixture categories is given below. In general, the use of the chemical category concept for chemical mixtures is not straightforward and further guidance will need to be developed as more experience is gained.

#### Isomers and their mixtures

40. Isomers are chemicals that have identical molecular formulas but different molecular arrangements. Although there are several types of isomers, the two that typically will be considered within the HPV Chemicals Programme are *structural* and *geometric*.

41. Structural isomers are molecules with differences in the arrangement of their atoms, such as butene-1 and isobutene. Structural isomers can include:

- chain isomers, for example hydrocarbon chains with identical or variable lengths and variable branching patterns
- position isomers, for example hydrocarbon chains with a functional group that varies in position along the chain

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<sup>6</sup> The concept applies to reaction products or process streams and does not refer to intentional mixtures of substances (or preparations).

42. A third type of structural isomer is referred to as a functional group isomer. These isomers also have identical molecular formula, but contain different functional groups. Examples of two functional group isomers with C<sub>4</sub>H<sub>10</sub>O as a molecular formula are 1-butanol and 2-butanol. Each of these isomers contain a hydroxyl group (C-O), but are representative of two different chemical families, alcohols and ethers. Although structural isomers, this type is less likely to be considered within a category for the Programme because functional isomers can have very different chemical and biological properties. Functional isomers are not included within the scope of this guidance.

43. Geometric, or stereo, isomers contain their molecules in the same arrangement, but a section or sections of each have different spatial arrangements. For example, *cis*-butene-2 and *trans*-butene-2 each have carbon groups on either side of a double bond, which cannot rotate, that are arranged on either the same side of the molecule (*cis*-) or opposite sides of the molecule (*trans*-).

44. Geometric and select structural isomers can have similar, somewhat different or very different chemical or toxicological properties. Even though they may behave identically in many chemical reactions, it is for example well known that the enzyme specificity in biological systems may be totally different and extreme caution is needed in case of such substances. An example of such specificity is select carbohydrates, which may be metabolised or not depending on the orientation of functional groups. Another example showing a profound difference in effects is the drug Thalidomide, which have one chiral atom and therefore exists as two enantiomers. The optical "R" isomer is an effective sedative and the optical "S"- isomer is a teratogen causing serious birth defects in children to mothers using the drug during pregnancy."

45. There are general rules for using read-across techniques as they apply to isomers:

- Relatedness - The substance(s) without data as well as the substance(s) with data are similar such that their physicochemical, biological, and toxicological properties would be expected to behave in a predictably similar manner or logically progress across a defined range.
- Structural Similarity - The substance(s) without data possesses a small incremental structural difference from the reference substance(s) or the difference between the two would not be expected to affect the property sufficiently such that it could not be accurately predicted.

46. There can be instances within a category of isomers, specifically as related to structural isomers, when read-across for an endpoint is not appropriate. An example is illustrated with two categories of isomers other than the butenes, the pentanes and hexanes. Though the pentanes may be broadly described as isomers, they actually represent three types of hydrocarbons, normal alkanes, branched alkanes, and cyclic alkanes. It is known that n-pentane, 2-methylbutane, 2,2-dimethylpentane, and cyclopentane exhibit distinct differences in potential biodegradability. n-Pentane and 2-methylbutane are readily biodegradable, whereas 2,2-dimethylpentane and cyclopentane are poorly biodegraded. Therefore, it would not have been possible to assess the biodegradability of the poorly biodegradable pentanes if they had no data using the results from the readily biodegradable pentanes even though the pentane isomers could still be considered a category for all other endpoints within the Programme. In such a case, the potential biodegradability of the two groups of pentanes would each have to be characterised separately within the context of the category. Likewise, the peripheral neurotoxicity in humans associated with n-hexane exposure has not been demonstrated to occur with exposure to other hexane isomers and a discussion of this effect within a hexane isomer category would have to isolate n-hexane from the other isomers.

47. An example of a category of isomers is provided in Annex 1 (Example D: Butenes and their mixtures). Based on this example, general principles of read-across/extrapolation and application of data within a category of isomers and their mixtures can include:

- Select properties of isomers may be read-across to another isomer(s) or to an isomeric mixture within a category if the data are similar and/or if the structure of the isomer(s) without data is similar to the isomers with data.
- Extrapolating properties to isomeric mixtures should take into account mode of action, potential additivity and synergy, as well as purity profiles, and mixture composition.
- For toxicological endpoints (e.g., LC<sub>50</sub>, NOAEL) a range of toxicity or the lowest value in a range of toxicity may be used for read-across.
- Read-across from one isomer to another may not be straightforward. Metabolic data may be needed if existing knowledge of category members or related non category members suggests that differences may be expressed within a biological endpoint of interest.

### Complex substances

48. Complex substances include a diverse range of materials which are frequently described as substances of *Unknown or Variable composition, Complex reaction products or Biological material* (UVCB Substances). There are many different types of complex substances, though generally they all have the following characteristics in common.

- They contain numerous chemicals (typically closely related isomers), and cannot be represented by a simple chemical structure or defined by a specific molecular formula. They are, however, assigned unique Chemical Abstract (CAS) numbers (see note<sup>7</sup> below about unique issues with CAS numbers for UVCB substances).
- They are not intentional mixtures of chemicals.
- Many are of natural origin (e.g., crude oil, plant extracts) and cannot be separated into their constituent chemical species.
- The concept of “impurities” typically does not apply to complex substances.

49. Category approaches for complex substances may vary, though generally the approach will be related to how the substances are manufactured, defined and used. For example, petroleum substances are generally defined by hydrocarbon chemistry (e.g., aliphatic hydrocarbons, aromatic hydrocarbons, etc.), physicochemical properties such as boiling range or carbon-number range, manufacturing and processing conditions, and common use categories. For hydrocarbon solvents [see example E provided in Annex 1], the categories are based on the typical chemistry and carbon-number range of hydrocarbon solvents and common uses. Under this approach, those hydrocarbon solvent substances with similar chemistry and

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<sup>7</sup> CAS numbers are important for identifying substances; however, for UVCB substances they do not represent a unique chemical and the specificity of the CAS number definition may vary (some CAS number definitions are rather narrow, some are very broad). CAS numbers for complex petroleum substances are based on a hierarchy of considerations including hydrocarbon type, carbon number range, distillation range and the last processing step. Because of these numerous considerations, similar products sometimes have different CAS numbers. There are also historical and geographical reasons why similar substances may have been assigned different CAS numbers. Further, some CAS numbers have a broad definition that may fit different substances that would fall into different categories. Because of this, physical properties and chemical structure are the preferred way to construct categories of complex substances.

carbon-number range are grouped together in the same category and the category is defined by the composition of those substances. This approach is practical and has the benefit of making sure that similar commercial products are grouped together in the same category.

50. Based on the example described in Annex 1, some general guidance can be provided for developing chemical categories with complex mixtures:

- It is important to clearly characterise mixtures, details of the production process can be useful. It is necessary to identify the following attributes of a complex mixture:
  - Composition (what is present and in what proportion)
  - Impurities (substances present that are not wanted but need to be identified)
- Properties of the components of a complex mixture can be applied to the complex mixture if the properties of the single components are similar.
  - It is necessary to identify representative components of the mixture to cover the carbon range and structures of the mixture.
  - Components with outlying properties need to be identified (e.g. specific toxicity of hexane compared to other aliphatic hydrocarbons, higher water solubility of aromatic hydrocarbons compared to aliphatic hydrocarbons).
- Properties of a complex mixture can be read-across to another complex mixture if the composition of the two are similar.
- Quantitative read-across is more difficult (ranges can be used where applicable). It is necessary to carefully consider the dose for read across because of the nature of the mixtures and the amount of components of concern.
- It is necessary to carefully identify representative substances for testing purposes.

#### Metal and metal compounds

51. The concept of chemical categories has traditionally been widely used for inorganic substances. However, not much experience is available to date of a systematic use of this approach. The concept is being used for the assessment of nickel and nickel compounds (see example in Annex 1).

52. There are a number of assumptions underlying any grouping of metal compounds for estimating their biological properties. The main assumption is that it is the metal ion that is responsible for the effects to be assessed. This is considered to be a reasonable assumption for the majority of the inorganic and some organic anions. This implies that in the case of inorganic salts, the toxicity of the counter ion is assumed to be largely irrelevant in producing the effects to be assessed. If the counter ion influences significantly the effects of the compound to be assessed, it can not be part of the category. Where a metal can have different valence states (e.g. chromium), the toxicities of the different valence states may vary, and the different valence states considered separately.

53. The water solubility of the metal compounds is often used as the starting point for establishing a category, as this reflects the availability of the metal ion in the different compartments of interest. For inorganic nickel compounds, a number of sub-groups have been suggested, reflecting different ranges of aqueous solubility. In contrast to inorganic nickel compounds it is not obvious how to group organic nickel compounds based on solubilities alone.

54. Based on the example of nickel and nickel compounds, some tentative general guidance for metal and metal compounds can be proposed:

- The main assumption is that the metal ion (or ion complex) is responsible for the effects to be assessed (the toxicity of the counter-ion is assumed to be largely irrelevant in producing the effects to be assessed).
- One basis of grouping could therefore be water solubility (inorganic metal compounds), taking into account:
  - transformation/ dissolution of insoluble compounds
  - bioavailability of the metal ion in the environment
  - solubility in biological fluids
  - persistence in the body

Other bases for grouping can be considered. For example if data is available, for systemic effects the solubility of the different compounds in the acidic environment of the stomach could be considered.

- The assumption that the metal ion (or ion complex) is mainly responsible for the effects rather than the counter-ion may not work for local mammalian toxic effects.
- Possible differences in the toxicity of different oxidation states of the metal ion (or ion complex) should be considered.
- Whilst the assumptions shown above can be expected to be valid for a wide range of inorganic compounds, these do not necessarily apply to organically based metal compounds. A different approach may be needed for grouping organic metal compounds. .

55. It should be noted that whilst this example considers groups of metallic cations, similar considerations would also apply to salts of anions where there are concerns for toxicity (e.g. cyanides, oxalates).

### **3.2.6 Experience in Developing Chemical Categories**

56. OECD experience provides a framework for handling categories. However, since that experience is limited, lessons learned in the OECD, and other similar programmes such as the US HPV Challenge Programme, will provide a measure of feedback and review.

57. The largest categories applied in the OECD HPV Chemicals Programme to date contain eight to ten chemicals. This is not a formal maximum, but acceptable categories will tend to be self-limiting because endpoint trends are generally disturbed as structural variations become more complex. Practically the analysis of large categories can also become unwieldy tending to limit the size of categories proposed. In this regard, groups of related individual categories may be considered, each one contributing elements in the design and implementation of an overall category strategy. A larger category may be justifiable in certain cases, such as when toxicity of the category is generally low.

58. Annex 1 contains a number of examples of how a category approach has been used for the purpose of collecting, reporting, and assessing hazard information in the OECD HPV Chemicals Programme. Other examples of categorising chemicals for hazard assessment purposes include the CONCAWE (the European oil company organisation for environment, health and safety) approach of categorising chemicals in petroleum streams (CONCAWE, 1998), approaches to assess the ecotoxicity (Bowmer et al., 1998) and health effects (Clary, et al., 1998) of lactate esters, and a number of category/SAR analyses by the German authorities (Greim, et al., 1994, 1995, 1998; and Poelloth and Mangelsdorf, 1997).

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## ANNEX 1

### EXAMPLES OF CATEGORY APPROACHES

1. Examples presented in this Annex are chemicals being investigated in the OECD HPV Chemicals Programme. They have been shortened for purposes of presentation in this document to illustrate the steps for identification and development of categories included in this guidance document. The examples are:

- A. Alpha-olefins - discrete chemicals with an incremental and constant change across the category;
- B. Linear alkyl benzenes - family of mixtures; and
- C. Brominated diphenyl ethers - family of congeners.
- D. Butenes – family of isomers and their mixtures
- E. Hydrocarbon solvents – family of complex mixtures
- F. Inorganic nickel compounds

#### Example A: Alpha Olefins Series

##### *Step 1: Identification of structure-based category and its members:*

2. The category was defined as olefins bearing a single medium-length, even-numbered, unbranched aliphatic chain with no other functional groups (“ $\alpha$ -Olefins”). This category consists of discrete chemicals with an incremental and constant change across its members (dimethylene group). Because the double bond is terminal, possible metabolic reactions such as oxidation at the double bond or allyl position should not be unduly affected by the chain lengthening. The lower ( $C_6$ ) and upper ( $C_{14}$ ) boundaries were based on the available product lines of the sponsors involved in the OECD effort.

3. The chemical structure of the category is:



R = CH<sub>3</sub>, n-Propyl, n-Pentyl, n-Heptyl, n-Nonyl

##### *Step 2: Gather published and unpublished literature for each category member.*

4. A literature search resulted in identifying a significant amount of available data for most category members in most of the major SIDS endpoints.

##### *Step 3: Evaluate available data for adequacy.*

5. Available data was evaluated at the individual study level and collected for each member of the category. Available data were compiled and included all SIDS endpoints and other relevant information; non-SIDS data were found and used in the hazard profile (e.g., aspiration hazard potential to humans).

##### *Step 4: Construct a matrix of data availability.*

6. Table A-1 is a matrix of SIDS endpoints and available/adequate data for each member of the alpha-olefin category. For simplicity, not all relevant data are presented.

##### *Step 5: Perform an internal assessment of the category.*

7. The information in Table A-1 identifies where data gaps exist (noted as “-“ in the table). Adequate data (noted as “√” in the table) are available for most endpoints. Endpoint data were evaluated to determine whether they correlate with chemical structure to judge the acceptability of the category. Although not shown in Table A-1, the data suggested that water solubility decreased with increasing chain length and aquatic toxicity appeared to decrease with increasing chain length.

<b>Table A-1</b>					
<b>STEP 4: Matrix of Available and Adequate Data on Alpha-Olefin Category Members</b>					
<b>Test</b>	<b>Hexene</b>	<b>Octene</b>	<b>Decene</b>	<b>Dodecene</b>	<b>Tetradecene</b>
<b>Physicochemical Properties</b>					
Partition Coeff.	√	-	√	√	-
Water Solubility	-	-	-	√	√
<b>Environmental Fate</b>					
Biodegradation	√	-	√	√	√
<b>Ecotoxicity</b>					
Acute Fish	√	-	√	√	-
Acute Daphnid	√	-	√	√	-
Alga	√	-	√	√	-
Terrestrial	-	-	√	-	-
<b>Human Health Effects</b>					
Acute Oral	√	√	√	√	√
Acute Inhalation	√	√	√	√	√
Acute Dermal	√	√	√	√	√
Repeated Dose	√	√	-	-	-
Genotoxicity (in vitro - bacteria)	√	√	√	√	√
Genotoxicity (in vitro - non-bacterial)	√	√	-	√	√
Genotoxicity (in vivo)	√	-	-	-	-
Repro/Developmental	-	-	-	-	-
(√) = Data available and considered adequate; (-) = No data available, or available data considered inadequate.					

**Step 6: Prepare category test plan.**

8. Table A-2 contains the proposed testing plan only for the endpoints for which new testing was recommended for the alpha-olefins. In this case it appears reasonable that if data gaps are filled by testing at the upper and lower ends of the homologous series (shaded regions in the table), and if the results suggest a pattern, then the remaining data gaps can be considered to fall within the ranges defined by the data.

**Step 7: Conduct necessary testing.**

9. The shaded cells in Table A-2 show where new testing was recommended for the category.

<b>Table A-2</b> <b>Alpha-Olefin Proposed SIDS Test Plan<sup>1</sup></b>					
<b>Selected SIDS Endpoint</b>	<b>Hexene</b>	<b>Octene</b>	<b>Decene</b>	<b>Dodecene</b>	<b>Tetradecene</b>
Water Solubility	√/-	-	-	√/+	√/+
Acute Fish	√/+	-	√/+	√/+	-
Acute Daphnid	√/+	-	√/+	√/+	-
Acute Algae	√/+	-	√/+	√/+	-
Repeated Dose	√/+	√/+	-	-	- <sup>2</sup>
Repro/Developmental	-	-	-	-	- <sup>2</sup>

<sup>1</sup> KEY: √/- = data available, but not adequate; √/+ = data available and considered adequate; - = no data available. Shaded cells represent those SIDS endpoints for which testing was recommended.

<sup>2</sup> A combined repeated dose and reproductive/developmental toxicity screen study design was recommended.

***Step 8: Perform an external assessment of the category.***

10. Table A-3 shows the results of the recommended testing and how it “fit” with available data for purposes of evaluating whether a pattern exists between some of the SIDS endpoints and the increase in 2-carbon increments from hexene to tetradecene. Note that there are four data points that exist in Table A-3 that were not present in Table A-2 (the octene water solubility and ecotoxicity results); these data were a late addition to the octene dossier and are included here to enhance the category analysis. This illustrates how all data should be considered in the evaluation of a category, even if it becomes available well after the literature search has been completed.

11. The new data show that patterns are clearly evident. For example, there is an apparent decrease in water solubility with increase in carbon chain length and a decrease in acute toxicity to fish and daphnids with an increase in carbon chain length. On the other hand, the mammalian toxicity data suggest a pattern of no difference between hexene and tetradecene for repeated dose (general) toxicity and developmental/ reproductive toxicity.

**Table A-3**  
**Results and Interpolation of Alpha-olefin SIDS Category Testing<sup>1</sup>**

Selected SIDS Endpoint	Hexene	Octene	Decene	Dodecene	Tetradecene
Water Solubility	50 mg/L <sup>2</sup>	(4.1 mg/L) <sup>3</sup>	INSOLUBLE	“insoluble”	0.0004 mg/L
Acute Fish	5.6 mg/L (LC <sub>50</sub> )	(4.8 mg/L) <sup>3</sup> (LC <sub>50</sub> )	>Water solubility? (Reported value >10,000 mg/L (LC <sub>50</sub> ))	>Water solubility? (Reported value >1000 mg/L (LC <sub>50</sub> ))	>Water solubility (LC <sub>50</sub> )
Acute Daphnid	10 mg/L (NOEC)	(3 < EC <sub>50</sub> > 10) <sup>3</sup>	>Water solubility? (EC <sub>50</sub> )	>Water solubility? (EC <sub>50</sub> )	>Water solubility (LC <sub>50</sub> )
Acute Algae	>Water solubility (LC <sub>50</sub> )	(>Water solubility) <sup>3</sup> (LC <sub>50</sub> )	>Water solubility? (EC <sub>50</sub> )	>Water solubility? (EC <sub>50</sub> )	>Water solubility (LC <sub>50</sub> )
Repeated Dose	NOEL <sub>oral</sub> = 101 mg/kg (males) and >1000 mg/kg (females)	NOEL = 50 mg/kg (males)	SIMILARLY TOXIC		NOEL <sub>oral</sub> = 100 mg/kg (males) and >1000 mg/kg (females)
Repro/ Developmental	NOEL <sub>repro</sub> and NOEL <sub>dev</sub> = >1000 mg/kg	SIMILARLY TOXIC			NOEL <sub>repro</sub> and NOEL <sub>dev</sub> = >1000 mg/kg

<sup>1</sup> KEY: - = no data available; shaded cells represent those SIDS endpoints for which OECD recommended testing.

<sup>2</sup> Apparently this was the original value thought not adequate, but estimations of the water solubility were similar to this value, so a new study was not performed.

<sup>3</sup> These data were not identified as being available in the Testing Plan. However, because they were reported in the dossier, they are included here to enhance the category analysis.

### **Step 9: Fill the data gaps**

Water solubility.

12. The 50 mg/L value for hexene and 0.0004 mg/L value for tetradecene suggest a wide range of solubility for the five members of the group. The octene value of 4.1 mg/L suggests that the pattern (decreasing water solubility with increasing chain length) holds. Therefore, water solubility tests were judged not necessary and computer estimates (consistent with the latter premise for decene and dodecene) were considered acceptable.

Acute aquatic toxicity

13. The data in Table A-3 suggests that hexene and octene may exhibit moderate acute toxicity to fish and daphnids based on measured values (NOEC, LC<sub>50</sub>, EC<sub>50</sub>). However, all other members of the category appear to show no effects on fish and daphnids at saturation. In the case of algae, all category members show no effects at saturation. From a category perspective, it appears that a declining pattern

exists for fish and daphnids (hexene and octene are more toxic than decene, dodecene, and tetradecene) but there was a flat pattern for algae (all members appeared equal). Based on this information, it was decided that no additional aquatic toxicity testing was necessary. The three literature values for octene noted in Table A-3 were considered acceptable. The aquatic acute toxicity for those endpoints correlate with water solubility, which in turn appear to determine (or limit) bioavailability of octene.

#### Repeated dose toxicity

14. The results presented in Table A-3 suggest that the general toxicity of hexene and tetradecene are similar, whereas octene appears more toxic than either hexene or tetradecene. In both cases, male rats were more sensitive than female rats. The effect observed in males, a male-rat specific kidney effect, does not appear to be relevant to humans. Also, both studies followed the OECD repeated dose/reproductive/developmental toxicity screening testing protocol. There were no data for either decene or dodecene. The octene data point suggests that any category pattern that might exist (equal toxicity across all members) given the hexene and tetradecene data might not exist for the middle members of the category. However, upon closer inspection of the octene data in the octene dossier, it is seen that the doses used in the repeated-dose study were 5, 50, and 500 mg/kg. Since the LOEL was 500 mg/kg, the “true” NOEL is anywhere from 50 to 500. Therefore, given these data, one could recommend that all members of the group likely have equal general toxicity under repeated dose conditions and testing of decene and dodecene is not required.

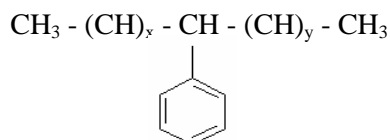
#### Reproductive/Developmental toxicity

15. The reproductive/developmental toxicity row in Table A-3 shows that data are available only for hexene and tetradecene. As with the repeated dose data, the results of the two studies were essentially the same. This suggests that it would not be necessary to test the middle three members of the category (octene, decene, and dodecene), especially given the results of the assessment of general toxicity (see above). The data suggest a consistent pattern across the category, or that all members are equally toxic for reproductive/ developmental effects under the conditions of the hexene/tetradecene studies (highest dose of 1000 mg/kg).

## Example B: Linear Alkylbenzenes

### Step 1: Identification of structure-based category and its members:

16. The linear alkylbenzene (LAB) category is comprised of nine different commercial formulations. Each formulation is a mixture containing various proportions of individual LABs with the following formulae:



Where  $x + y = 7-13$  and  $x = 0-7$ , giving a linear carbon range of  $\text{C}_{10}$  to  $\text{C}_{16}$ .

17. Thus, this category would fall under “family of mixtures” in terms of category type. Table B-1 presents the nine commercial products evaluated. Note that the LAB category may be further subdivided into three subcategories based on the percentage of alkyl substituents with a low ( $\text{C}_{10}$ - $\text{C}_{11}$ ), mid ( $\text{C}_{11}$ - $\text{C}_{13}$ ), and high ( $\text{C}_{13}$ - $\text{C}_{14}$ ) proportion of carbon chain lengths.

<b>Table B-1</b> <b>Assignment of LAB SubCategories<sup>1</sup></b>					
LAB Formulation	Carbon Chain Length for Substituted Alkyl Group (Numbers represent percent of total)				
	$\text{C}_{10}$	$\text{C}_{11}$	$\text{C}_{12}$	$\text{C}_{13}$	$\text{C}_{14}^{(2)}$
Nalkylene 500	21	39	31	7	<1
Nalkylene 500L	20	44	31	5	<1
<b>Alkylate 215</b>	16	43	40	1	<1
Nalkylene 550L	14	30	29	20	7
<b>Alkylate 225</b>	7	25	48	19	1
Nalkylene 575L	9	17	20	30	15
Nalkylene 600	<1	1	23	50	25
Nalkylene 600L	<1	1	23	50	25
<b>Alkylate 230</b>	1	2	16	50	30

<sup>1</sup> The two shaded regions and the open area make three subcategories by presenting two ends of the spectrum in terms of a higher proportion (>50%) of shorter carbon chains (upper left) and a higher proportion (>50%) of longer carbon chains (lower right). Bolded formulations had available data in all SIDS categories.

<sup>2</sup> The proportion of  $\text{C}_{15}$  and  $\text{C}_{16}$  is < 1% in all formulations except for an incidence of 1%  $\text{C}_{15}$  in Alkylate 230.

**Step 2: Gather published and unpublished literature for each category member.**

18. A literature search resulted in identifying data for most category members in the environmental fate, ecotoxicity and human health effect SIDS endpoints.

**Step 3: Evaluate available data for adequacy.**

19. Again, as was discussed in the alpha-olefin example, evaluation of data adequacy is performed at the individual study level. [Guidance for Determining the Quality of Data for the SIDS Dossier (Interim SIDS Manual)]

**Step 4: Construct a matrix of data availability.**

20. An analysis of available data resulted in a matrix as presented in Table B-2. Again, for simplicity not all data found or compiled are presented here. Note that three LAB formulations (Alkylate 215, Alkylate 225, and Alkylate 230) had data available in each of the major SIDS classes (environmental fate, ecotoxicity, and health effects), and they each represent one of the three subcategories presented in Table B-1.

<b>Table B-2</b>								
<b>STEP 4: Matrix of Available and Adequate Data on LAB CategoryMembers<sup>1</sup></b>								
LAB Formulation	Environmental Fate	Ecological Effects			Human Health Effects			
		Fish Acute	Daphnid Acute	Daphnid Chronic	Acute <sup>4</sup>	Repeated Dose <sup>5</sup>	Mutagenicity <sup>6</sup>	Developmental <sup>7</sup>
Nalkylene 500	-	-	-	-	√	-	-	-
Nalkylene 500L					-			
<b>Alkylate 215</b>	√	√	√	√	√	-	√	√
Nalkylene 550L	-	-	-	-	√	-	-	-
<b>Alkylate 225</b>					√			
Nalkylene 575L	-	-	-	-	-	-	-	-
Nalkylene 600								
Nalkylene 600L	-	-	-	-	√	-	-	-
<b>Alkylate 230</b>					√			

<sup>1</sup> “√” denotes data are available and adequate. “-“ denotes data are either not available, or are available and are judged inadequate. Shaded areas mark the three subcategories identified in Table B-1.

***Step 5: Perform an internal assessment of the category.***

21. As with Table A-1 in the alpha-olefin example, the data in Table B-2 identifies where data gaps exist. Note that adequate data are available for most endpoints for the three LAB formulations mentioned above. Table B-3 is essentially the same table as Table B-2, except that the data values are placed in each cell so that they can be evaluated to determine the acceptability of the category approach for each endpoint.

22. Table B-3 shows a consistent pattern of no discernible difference in aerobic degradation among the three LAB formulations tested (range of 56% - 61% of parent material evolved as carbon dioxide after a 35 day incubation period). Similarly, the acute fish toxicity, chronic daphnid toxicity, acute mammalian toxicity, reproductive/developmental toxicity, and mutagenicity data do not show differences across the tested formulations. However, the acute daphnid toxicity results, as well as the repeated dose toxicity tests in mammals suggest a pattern of increasing toxicity with an increase in the proportion of higher length carbon chains in the substituted alkyl group that appears to hold for each of these SIDS endpoints.

***Step 6, 7 and 8: Prepare category test plan for review; Conduct necessary testing; and Perform an external assessment of the category.***

23. In this case, it was concluded that no further testing was necessary under the SIDS programme and that the existing data were sufficient for a screening level hazard assessment. Thus; it was not deemed necessary to test each LAB formulation given the results of testing in three separate formulations to represent the boundaries of the category.

24. In this example, the test plan would include the rationale for “no testing” together with an evaluation of the existing data. Robust summaries for the individual supporting studies would also be available.

**Table B-3**

<b>Evaluation of Matrix Data Patterns for LAB Category</b>									
LAB Formulation	Environmental Fate	Ecological Effects			Human Health Effects				
		Fish Acute	Daphnid Acute	Daphnid Chronic	Acute <sup>4</sup>	Repeated Dose <sup>5</sup>	Mutagenicity <sup>6</sup>	Developmental <sup>7</sup>	
Nalkylene 500	Not tested		Not tested		>34 g/kg		Not tested		
Nalkylene 500L					Not tested				
<b>Alkylate 215</b>	<b>56%<sup>1</sup></b>	<b>&gt; Water solubility</b>	<b>80 ppb<sup>2</sup></b>	<b>7.5 to 15 ppb<sup>3</sup></b>	<b>17 g/kg</b>	<b>100 mg/m<sup>3</sup></b>	<b>Negative</b>	<b>125 mg/kg</b>	
Nalkylene 550L	Not tested		Not tested		>5 g/kg		Not tested		
<b>Alkylate 225</b>	<b>61%<sup>1</sup></b>	<b>&gt; Water solubility</b>	<b>9 ppb<sup>2</sup></b>	<b>Not tested</b>	<b>28 g/kg</b>	<b>29 mg/m<sup>3</sup></b>	<b>Negative</b>	<b>Not tested</b>	
Nalkylene 575L	Not tested		Not tested				Not tested		
Nalkylene 600	Not tested		Not tested		>35 g/kg		Not tested		
Nalkylene 600L					>5 g/kg				
<b>Alkylate 230</b>	<b>56%<sup>1</sup></b>	<b>&gt; Water solubility</b>	<b>10 ppb<sup>2</sup></b>	<b>13 to 23 ppb<sup>3</sup></b>	<b>21 g/kg</b>	<b>&lt;32 mg/m<sup>3</sup></b>	<b>Negative</b>	<b>125 mg/kg</b>	

<sup>1</sup> Percent of parent material evolved as carbon dioxide after 35 days in an aerobic biodegradation test.

<sup>2</sup> 48-hour LC<sub>50</sub>s.

<sup>3</sup> 21-Day No Observed Effect Concentration (NOEC).

<sup>4</sup> Oral LD<sub>50</sub>s in rodents.

<sup>5</sup> Four week inhalation studies in rats, values represent NOECs for the following effects: irritation of the eyes and nose and decreased body weight.

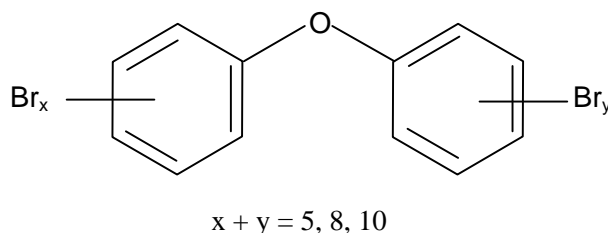
<sup>6</sup> Negative in vitro (bacteria - Ames; mammalian - Chinese hamster ovary cells) and in vivo (chromosomal aberration study in rats) tests.

<sup>7</sup> Developmental toxicity study (oral, rats, doses of 0, 125, 500, and 2000 mg/kg/d). Numbers in column represent no observed adverse effect level (NOAEL) for both maternal (weight gain) and developmental (ossification variations) endpoints.

### Example C: Brominated Diphenyl Ethers

#### Step 1: Identification of structure-based category and its members:

25. The polybrominated diphenyl ether (PBDE) category theoretically contains a number of congeners (mono- through to decabromodiphenyl ether) but only three products are produced commercially: bis (pentabromophenyl) ether, also known as decabromodiphenyl ether (decaBDE); diphenyl ether, octabromo derivative (octaBDE); and diphenyl ether, pentabromo derivative (pentaBDE). The general chemical formula for this category is:



26. This category is the “family of congeners” category type and is limited to three products produced commercially as most of the laboratory test data has been obtained with these products. DecaBDE is an essentially pure substance, but the other two are complex mixtures of related substances with varying degrees of bromination and substitution patterns. The actual compositions of the commercial products are summarised in table C-1. This particular example is limited to an analysis of ecotoxicity data.

Congener	PentaBDE	OctaBDE	DecaBDE
tribromo-	0-1%		
tetrabromo-	24-38%		
pentabromo-	50-62%	1.4-12% <sup>1</sup>	
hexabromo-	4-12%		
heptabromo-	trace	43-58%	
octabromo-		26-35%	
nonabromo-		8-14%	≤3%
decabromo-		0-3%	≥97%

<sup>1</sup>This figure refers to the combined total of pentabromo- and hexabromo- congeners present.

#### Step 2: Gather published and unpublished literature for each category member.

27. A literature search identified some ecotoxicity data for all category members.

#### Step 3: Evaluate available data for adequacy.

28. As with the other examples in this Appendix, evaluation of data adequacy is performed at the individual study level.

**Step 4: Construct a matrix of data availability.**

29. Table C-2 presents a matrix for the available ecotoxicity data based on the literature search. SIDS data gaps exist for acute invertebrate testing (decaBDE) and for acute algal testing (octaBDE).

<b>Table C-2</b>			
<b>STEP 4: Available Acute Ecotoxicity Data on PBDEs<sup>1</sup></b>			
Test Organism	PentaBDE	OctaBDE	DecaBDE
Fish	√	√	√
Invertebrate	√	√	-
Algae	√	-	√

<sup>1</sup> “√” denotes data available and adequate; “-” denotes data not available, or available and not adequate.

**Step 5: Perform an internal assessment of the category.**

30. Table C-3 is essentially the same table as Table C-2, except that actual data replace the “√s”.

31. In evaluating these data, it was concluded that a decrease in aquatic toxicity could be expected with increasing bromine number. Since there were adequate aquatic toxicity data for the category member with the lowest number of bromine atoms (pentaBDE), it was not necessary to conduct additional acute toxicity tests on the remaining members with a higher number of bromine atoms. DecaBDE would not be more toxic to invertebrates than octaBDE, and the algal toxicity of octaBDE could be inferred from the data on penta- and decaBDE.

32. In addition to the ecotoxicity data, available data on environmental monitoring, bioconcentration, and the physicochemical properties of the category members were evaluated. It was determined that there was a decreasing concern for bioaccumulation potential with an increase in bromine number; that all three compounds were not very water soluble; and that they all had high octanol-water partition coefficients (log K<sub>ow</sub>). This suggested that the likely exposure scenario of concern would be to organisms exposed directly to sediment or soil.

<b>Table C-3</b>			
<b>Available Acute Ecotoxicity Data on PBDEs</b>			
Test Organism	PentaBDE	OctaBDE	DecaBDE
Fish	Rainbow trout NOEC (96 hr) = 21 µg/L (>water solubility?)	Medaka LC <sub>50</sub> (48 hr) = >water solubility	Medaka LC <sub>50</sub> (48 hr) = >water Solubility
	Medaka LC <sub>50</sub> (48 hr) = > water solubility		
Invertebrate	Daphnid EC <sub>50</sub> (48 hr) = 14 µg/L NOEC (48 hr) = 4.9 µg/L (EC <sub>50</sub> values close to water solubility)	Daphnid 21-day NOEC > 2 µg/L	No Data
Algae	<i>Selanastrum capricornutum</i> NOEC (96 hr) up to 26 µg/L (>water solubility?)	No Data	Three different species EC <sub>50</sub> (72 hr) > water solubility

<sup>1</sup> Small freshwater fish (warm water species).

**Step 6: Prepare category test plan for review.**

33. Because of the concern for bioaccumulation and partitioning of the PBDEs to the sediment/soil environment, it was recommended that further testing (chronic aquatic toxicity, sediment toxicity, and soil toxicity) be conducted, beginning with pentaBDE. Therefore, the final testing recommendation required “advanced” SIDS testing without filling the acute aquatic toxicity basic SIDS data gaps. The testing plan (Table C-4) was tiered, the results of the lower tiers determining the next set of tests.

Tier	Category Member	Ecotoxicity Test <sup>1</sup>	Result	Comment
I	PentaBDE	Fish early life stage test	Rainbow trout 60-day NOEC = 8.9 µg/l	Fish test to verify bioaccumulative potential.
		Daphnid reproduction test	Daphnid 21-day NOEC = 5.3 µg/l	Daphnid study to verify that acute effects were due to toxicity.
		Sediment (midge) toxicity test	<i>Chironomus riparius</i> 28-day NOEC = 16 mg/kg dry weight	To verify concerns identified in hazard/exposure assessment
		Sediment (oligochaete) toxicity test	<i>Lumbriculus variegatus</i> 28-day NOEC = 3.1 mg/kg dry weight	
		Sediment (amphipod) toxicity test	<i>Hyaella azteca</i> 28-day NOEC ~ 6.3 mg/kg dry weight	
		Soil (earthworm) toxicity test	<i>Eisenia fetida</i> 14-day NOEC >500 mg/kg dry weight	
		Soil (plant) toxicity test	Six plants – lowest 21-day EC <sub>5</sub> = 16 mg/kg dry weight	
		Soil (nitrification inhibition) toxicity test	Soil microorganisms 28-day NOEC ≥ 1 mg/kg dry weight	
II	OctaBDE	Sediment (oligochaete) toxicity tests using two sediment types	<i>Lumbriculus variegatus</i> 28-day NOEC ≥ 1,272 mg/kg dry weight	Tests were chosen based on the pentaBDE results – for example, sediment organism sensitivity is not expected to differ significantly and so only the most sensitive organism from the pentaBDE test series required testing.
		Soil (earthworm) toxicity test	<i>Eisenia fetida</i> 56-day NOEC ≥ 1,470 mg/kg dry weight	
		Soil (plant) toxicity test	Six plants – 21-day NOEC ≥ 1,190 mg/kg dry weight	
III	DecaBDE	Sediment (oligochaete) toxicity tests using two sediment types	<i>Lumbriculus variegatus</i> 28-day NOEC ≥ 3,841 mg/kg dry weight	As for octaBDE
		Soil (earthworm) toxicity test	<i>Eisenia fetida</i> 56-day NOEC ≥ 4,910 mg/kg dry weight	
		Soil (plant) toxicity test	Six plants – 21-day NOEC ≥ 5,349 mg/kg dry weight	

<sup>1</sup> All tests are beyond the basic SIDS requirements. The testing plan is presented to show how basic SIDS requirements were waived in order to proceed to a more meaningful testing scheme.

***Step 7. Conduct necessary testing.***

34. The results of the testing plan are summarised in Table C-4.

***Step 8. Perform an external assessment of the category.***

35. The Tier I testing was carried out using pentaBDE alone and consisted of long-term toxicity tests with exposure via water (tests with daphnids and rainbow trout), sediment (tests with midges, oligochaetes and amphipods) and soil (tests with earthworms, plants and soil microorganisms). The results of these tests verified concerns over the toxicity of the substance to fish and daphnids over long-term exposures, and confirmed that the substance could also elicit toxic effects on sediment- and soil-dwelling organisms. The Tier II (octaBDE) and Tier III (decaBDE) testing was actually performed in parallel, based on the most sensitive soil and sediment organisms identified in the Tier I testing.

36. In addition to the prescribed test plan, new information also became available on the accumulation potential of one member of the category, namely decaBDE. This showed that decaBDE was present in certain predatory birds and their eggs, as well as certain terrestrial mammals. Before these data were available it was assumed that decaBDE had a very limited potential for uptake and accumulation in organisms because of its large molecular size and low fish bioconcentration factor. In addition, new data also became available that appeared to show that the substance may cause neurotoxic effects in young laboratory mice at doses much lower than those causing effects for other toxicity endpoints (although further testing is currently underway to confirm this effect and establish a NOAEL). These new data required the assumptions over the low accumulation and hazard potential of decaBDE to be re-evaluated.

***Step 9: Fill the data gaps***

37. A major problem encountered with this category was that two members (octa- and decaBDE) showed effectively no toxicity in any of the aquatic (water exposure), sediment or soil toxicity tests undertaken. For aquatic toxicity it was possible to read-across between the various members of the category to fill the data gaps once the further testing for pentaBDE was completed, as follows. All three category members showed essentially no toxicity to fish from acute exposures. Penta- and decaBDE showed essentially no toxicity to algae, and so it was possible to infer that octaBDE would also show essentially no toxicity to algae. The long-term toxicity data for pentaBDE showed that invertebrates were the most sensitive trophic level. As octaBDE had no effect on daphnids following long-term exposure at concentrations up to its solubility limit, it was also possible to infer that decaBDE would show no effects with daphnids. Furthermore, both octa- and decaBDE were also considered unlikely to show any long-term effects in fish tests at concentrations up to their respective solubility limits (since both have a lower fish bioconcentration factor than pentaBDE).

38. In retrospect, it would have been possible to read-across from the octaBDE data to predict that decaBDE would also show little or no toxicity to soil and sediment organisms. However, at the time it was not possible to carry out this read-across with any certainty; regulatory experience with these types of tests is less extensive than for aquatic tests (for industrial chemicals), and the possible exposure in sediment and soil is not necessarily limited by the water solubility of the substances (oral exposure could be important). There was also a policy need to complete the testing as quickly as possible, and so the testing on both octaBDE and decaBDE was performed at the same time.

39. It should be noted that the main driver for the further testing requirements of octa- and decaBDE was consideration of the main compartments through which organisms may be exposed (i.e. sediment and soil) rather than to complete the SIDS endpoints (mainly related to exposure through water).

40. Overall, read-across of data was compromised by the fact that two members out of three essentially showed no toxicity in standard tests. The number of members in the category is an important consideration because it is very difficult to reliably identify trends from two data points (for example, it would generally not be possible to predict the behaviour of pentaBDE from that of octa- or decaBDE). The patterns and trends in the data for this category only really became evident once a substantial amount of testing had been carried out. However, it was useful to identify the most sensitive species in tests with the most toxic member of the category, which could then be used to target the testing for the other members of the category.

41. Another problem encountered with this category was that, with the exception of decaBDE, the commercially supplied substances contain a mixture of congeners. Much of the available ecotoxicity information was obtained with these commercial products and these data did not allow the actual toxicity of the individual congeners to be ascertained, except for the broad trends. This contrasts with the situation regarding the data on the environmental exposure of this group of substances, where a large database of monitoring data relating to specific isomers is available.

42. Perhaps most importantly for this category, it was not possible to identify or infer the particular accumulative properties of decaBDE using a read-across approach. For example, the available laboratory studies show that pentaBDE has a much higher accumulation potential than decaBDE and this is also demonstrated by the majority of the available environmental monitoring data (particularly for the aquatic environment). However, it has recently become apparent that decaBDE can be widely found in certain predatory birds and their eggs and some predatory mammals, possibly linked to the terrestrial food chain. This finding means that it is necessary to re-visit the category assumptions when new information on a category member is made available.

## Example D: Butene Isomers and their Mixtures

### Step 1: Define the category

43. An example of a category of isomeric substances and their mixtures is the butenes. This category includes four isomers (two structural isomers, butene-1 and isobutene), two geometric isomers, *cis*-butene-2 and *trans*-butene-2 and two mixtures (see table D-1) Substance structures are shown in Figures D-1 to D-4.

Figure D-1. Molecular Structure of Butene-1 (C<sub>4</sub>H<sub>8</sub>)

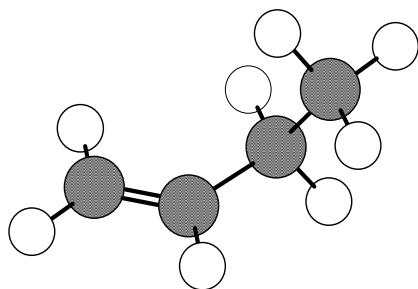


Figure D-2. Molecular Structure of Isobutene (2-methyl propene) (C<sub>4</sub>H<sub>8</sub>)

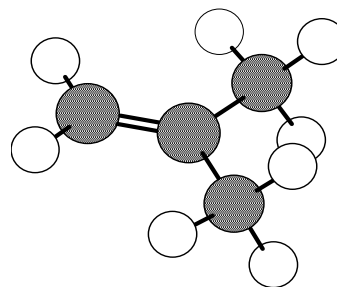


Figure D-3. Molecular Structure of *cis*-Butene-2 (C<sub>4</sub>H<sub>8</sub>)

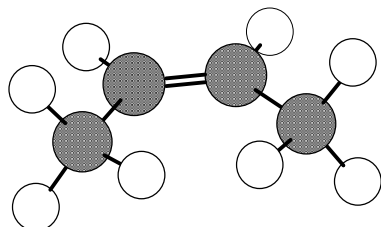
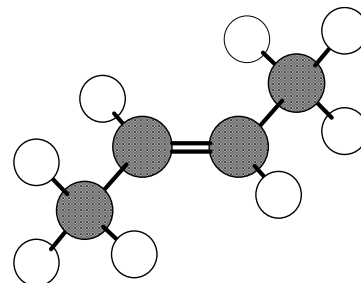


Figure D-4. Molecular Structure of *trans*-Butene-2 (C<sub>4</sub>H<sub>8</sub>)



44. Each butene isomer contains four carbon and eight hydrogen atoms, with one double bond between carbon number one and two or carbon number two and three. These are simple hydrocarbons in a class referred to as alkenes. At standard temperature and pressure (STP), they are gaseous.

45. Butenes are a good example of an isomer category because their physicochemical and biological properties are closely aligned. Based on existing data for toxicological endpoints within the HPV Chemicals Programme and the overall toxicological knowledge of hydrocarbons, the Butenes Category members that do not have toxicological data would be expected to exhibit similar if not identical effects as category members with data (the toxicological endpoints under consideration include mammalian and environmental as specified under the Programme). Additionally, because of their similarity with respect to physicochemical and biological parameters, data on the individual substances can be used to estimate values for the mixtures.

46. It should be noted that the Butenes Category example is relatively simple with a limited number of isomers. As the number of carbon atoms and functional moieties increase, the number of isomers increases illustrating the importance of appropriately defining isomer categories when optimising the use of existing data.

**Step 2: Gather published and unpublished data for each category member**

47. A comprehensive literature search identified information on all SIDS endpoints for the category members. The majority of information was found on the single isomers rather than on the mixed isomers. Participating companies in the consortium were asked to review their internal files for appropriate information.

**Step 3: Evaluate available data for adequacy**

48. Each study identified by the literature search was reviewed and assessed for quality and reliability (section 3.1). Data were entered into IUCLID 4.01 under the specific CAS number for each the isomers or as mixture as part of a "Butenes Isomers and their mixtures", category record. Those studies that were considered appropriate were classified as "Critical study for SIDS Endpoint". For several health related endpoints, more than one "critical study" was identified.

**Step 4: Construct a matrix of data availability**

49. The IUCLID category matrix report was used to automatically generate tables containing substance identity, end point, data availability and their values. The table is not reproduced here to save space, but a summary is given in Table D-1.

**TABLE D-1**

<b>Matrix of available and adequate data on butenes: isomers and their mixtures</b>						
<b>Test</b>	<b><i>1-Butene</i></b>	<b><i>Butene, mixed -1 and -2-isomers</i></b>	<b><i>2-Methylpropene</i></b>	<b><i>Butene, mixed isomers</i></b>	<b><i>cis-2-Butene</i></b>	<b><i>trans-2-Butene</i></b>
	106-98-9	107-01-7	115-11-7	25167-67-3	590-18-1	624-64-6
<b>Physicochemical Properties</b>						
Melting Point	√	–	√	–	√	√
Vapour Pressure	√	–	√	–	√	√
Partition Coeff.	√	–	√	–	√	√
Water Solubility	√	–	√	–	√	√
<b>Environmental Fate</b>						
Biodegradation	–	–	–	–	–	–
<b>Ecotoxicity</b>						
Acute Fish	√	–	√	–	√	√
Acute Daphnid	√	–	√	–	√	√
Alga	√	–	–	–	√	√
Terrestrial	√	–	√	–	√	√

Human Health Effects						
Acute Oral	-	-	-	-	-	-
Acute Inhalation	-	√	√	-	-	-
Acute Dermal	-	-	-	-	-	-
Repeated Dose	√	√	√	-	-	-
Genotoxicity ( <i>in vitro</i> )	√	√	√	-	-	-
Genotoxicity ( <i>in vitro</i> - non-bacterial)	-	√	√	-	-	-
Genotoxicity ( <i>in vivo</i> )	√	-	√	-	-	-
Repro/Developmental	√	√	-	-	-	-

(√) = Data available and considered adequate; (-) = No data available, or available data considered inadequate.

50. As butenes are gaseous at STP the data for environmental toxicity are calculated values, using the ECOSAR program. Human health data are available for all endpoints, with a minimum of two category members having reliable data.

**Step 5: Perform an internal assessment of the category**

51. The information in Table D-1 was assessed for trends. For environmental toxicity data, values were calculated for each endpoint. These values were similar for each member, and are therefore considered to be applicable to the mixed isomer category members, for which it is not possible to calculate values.

52. For the health endpoints, where there were data, they were similar for all category members within the considerations of study variability. Table D-2 shows selected data from the IUCLID category report.

**TABLE D-2**

Endpoint	Substances					
	1-Butene	Butene, mixed -1 and -2-isomers	2-Methylpropene	Butene, mixed isomers	cis-2-Butene	trans-2-Butene
	106-98-9	107-01-7	115-11-7	25167-67-3	590-18-1	624-64-6
Acute Inhalation Toxicity		LC50: male/female rat > 10057 ppm for 4 hour(s)	* LC50: rat = 620 mg/l (270000ppm) for 4 hour(s)			
Repeated Dose Toxicity	Male/female rat; other: vapour inhalation; 28 days; 6 hours/day, 7 days/week; Doses: 0, 500, 2000 and 8000 ppm; Method: OECD	Male/female rat; inhalation; 2 weeks NOAEL: = 2500 ppm	Male/female rat; inhalation; 13 weeks NOAEL: 8000 ppm *			

	combined study TG422; NOAEL: = 8000 ppm					
Genetic Toxicity <i>in vitro</i>	* Salmonella negative	* Salmonella negative	* Mouse lymphoma L5178Y TK+TK- negative			
Genetic Toxicity <i>in vivo</i>	Mammalian Bone Marrow Erythrocyte Micronucleus Test Negative		Micronucleus assay mouse negative			
Toxicity to Reproduction	OECD Guide- line 422 parental NOAEL = 8000 ppm	OECD 422 NOAEL Maternal toxicity: = 2500 ppm; NOAEL Teratogenicity: 5000 ppm				
Developmental Toxicity / Teratogenicity	OECD Guide- line 422 NOAEL parental: = 2500 ppm; F1: >= 5000 ppm		OECD 414 NOAEL Maternal toxicity: > 8000 ppm; NOAEL Teratogenicity: > 8000 ppm			

\* Endpoint where there was more than one study identified as a "critical" study. For the sake of this illustration only one study is shown. Multiple Endpoint data were not conflicting with data shown.

**Step 6: Prepare category test plan**

53. No testing was proposed.

**Step 7: Conduct the necessary testing**

54. No testing was proposed.

**Step 8: Perform an external assessment of the category**

55. No new information was generated so this step was not performed.

**Step 9: Fill data gaps by read-across, extrapolation, interpolation etc**

56. Read-across techniques can be applied to pure isomers within a category. For example, the log octanol-water partition coefficient ( $\log P_{ow}$ ) of *cis*-butene-2 is 2.3. If  $\log P_{ow}$  data were not available for *trans*-butene-2, based on the structural similarity, the data for *cis*-butene-2 could be used to estimate the  $\log P_{ow}$  of *trans*-butene-2, e.g.  $\log P_{ow} = 2.3$ . Selected properties of the pure isomers can also be used to

characterise a mixture containing the isomers. Using butene isomer log  $P_{ow}$  data as an example, if only butene-1 and isobutene data were available, 2.4 and 2.3, respectively, those data could be used to characterise the log  $P_{ow}$  of a substance containing all the butene isomers. The log  $P_{ow}$  value for such a substance could be represented as 2.3 to 2.4.

57. Although the previous discussion focused on a physicochemical endpoint, extrapolation of environmental and mammalian toxicity data are also possible from category members with data to those without data. It was possible to calculate select acute aquatic toxicity endpoints, including those within the Programme. However, if that had not been possible, the acute fish toxicity data for *cis*-butene-2 could have been used to estimate the toxicity of *trans*-butene-2. The appropriateness of this read-across would be justified based on the knowledge that the mode of toxic action for hydrocarbons is non-polar narcosis and that the toxic mechanism is disruption of biological membrane function. Therefore, because these substances would exert a similar biological effect and they would be expected to do so over a relatively narrow range. Based on their similar log  $P_{ow}$  values, an effect value (i.e., 96-hour fish LC50) for one butene isomer could be used to estimate the toxicity of another (the calculated fish toxicity values for the butene isomers range from 17 to 21 mg/L). Equally, if acute fish toxicity data were available for *cis*-butene-2, those data could be used to characterise the toxicity of a substance that contained *cis*-butene-2 and *trans*-butene-2.

58. An example of read-across as it can be applied to human health endpoints includes the application of repeated dose toxicity studies for three of the substances in this category (butene-1, isobutene, and butene mixed -1 and -2 isomers) to characterise the three substances without data (*cis*-butene-2, *trans*-butene-2, and butene mixed isomers). The three substances with data clearly indicate that they have a low order of subchronic toxicity. Adaptive and reversible changes in the liver indicate butenes were widely distributed within the body and metabolised. No Observed Adverse Effect Levels (NOAEL) from studies for tested members over 28-days to 2 years range from 2,000 ppm to 8,000 ppm. By read-across, the untested butene substances would also be expected to demonstrate a low order of subchronic toxicity over the extent and within the range to which the tested butenes were subjected, or to the lowest concentration of the range at a minimum.

59. Members of the Butenes Category that have been tested do not produce mutagenic responses either in *in vitro* or *in vivo* test systems. Butene-1, butene 1 and 2 mixed isomers, and isobutylene did not induce gene mutations in reverse mutation assays conducted in *S. typhimurium* and/or *E. coli* either in the presence or absence of metabolic activation. Butene-2 was not clastogenic to rat lymphocytes *in vitro*. Isobutylene tested negative in an *in vitro* cell transformation assay using a mouse embryo fibroblast derived cell line and in a mouse lymphoma assay both in the presence or absence of metabolic activation. In addition, neither 1-butene nor isobutylene induced micronuclei formation in mouse bone marrow cells. By read-across, these data support characterising the untested members of the Butenes Category as also having a low potential for carcinogenicity.

60. Inhalation reproductive/developmental toxicity studies conducted with butene-1, butene-2 mixed isomers, and isobutylene resulted in a NOAEL for each study that was the highest exposure concentration tested (5,000 to 8,000 ppm). Based on the weight of the experimental evidence and the consistent absence of observed significant toxic findings, the untested members of the Butenes Category would be expected to have a low potential for chronic, reproductive, and/or developmental toxicity and cancer.

61. Supportive evidence to consider the butenes as a category for mammalian toxicity endpoints is provided by an understanding of metabolism for selected butenes and their mode of action. Additionally, physicochemical data add to an overall understanding of the potential for distribution and data confirm that isomer form does not change toxicity. Metabolism of isobutylene via cytochrome P450 to an epoxide,

2-methyl-1,2-epoxypropane (MEP), has been demonstrated, and MEP has been identified as the primary metabolite in liver tissue of various species, including man. Epoxidation of *cis*- and *trans*-butene-2 has also been demonstrated. It is known that inhalation of these substances, only at very high concentrations, can produce central nervous system depression, anaesthesia and/or asphyxiation. For example, isobutylene is predicted to produce narcosis in man at concentrations exceeding the lower explosion limit of 18,000 ppm. Other butene substances would therefore also be expected to exhibit a similar low order of acute toxicity. Both the log  $P_{ow}$  and water solubility favour absorption via the lung and their small molecular weight and log  $P_{ow}$  suggest that butenes are likely to be widely distributed within the body. From data on tested butenes, it can be concluded that branching does not affect the toxicity of butenes. Evaluation of this information provides further support beyond the base toxicity data that butene substances would behave similarly in mammalian systems and exert similar degrees or lack of effects.

## Example E : HYDROCARBON SOLVENTS

[Note: The categories for Hydrocarbon Solvents have not been assessed by SIAM yet. The description below is based on the preparatory work of the sponsor organisations (International Hydrocarbon Solvents Consortium and US-EPA), as well as the discussions held at the OECD workshop on the development and use of chemical categories held in January 2004. The descriptions below might have to be revised once the categories have undergone assessment at SIAM.]

### Background

62. Hydrocarbon solvents assessed under the OECD HPV Chemicals Programme include aliphatic hydrocarbon solvents in the C5-C20 range and aromatic hydrocarbon solvents in the C9-C12 range. Production of hydrocarbon solvents is differentiated from other refinery substances such as gasoline and diesel fuel by additional processing steps leading to finished substances with narrow distillation range, a defined aromatic content, removal of benzene, polyaromatic hydrocarbons (PAHs), sulfur- and nitrogen-containing compounds, and low color. These additional refining steps are necessary in order to make substances with qualities suitable for consumer applications. The specific isomer content of most hydrocarbon solvents is not specified as these products are complex substances, not preparations or intentional mixtures, and may contain dozens or even hundreds of individual isomers. However, these substances are well characterized for their general chemical composition in terms of their paraffin, cycloparaffin, and aromatic content. In addition, the composition of specific constituents of concern (e.g., n-hexane, naphthalene) is well defined.

#### *Aliphatic Hydrocarbon Solvents*

63. Aliphatic hydrocarbons consist of carbon and hydrogen molecules arranged as straight chain (n-paraffins or n-alkanes), branched chain (isoparaffins), or cyclic hydrocarbons (naphthenes). They contain no reactive functional groups (e.g., alkenes, sulfides, alcohols, etc.). Commercial aliphatic hydrocarbon solvents can be individual hydrocarbons (e.g., n-pentane) or multi-constituent combinations of aliphatic hydrocarbons. These multi-constituent hydrocarbon solvents include products that are composed of one type of hydrocarbon chemistry (e.g., isoparaffins) or multiple types (e.g., white spirits, Varnish Makers and Painters (VM&P) Naphtha). Multi-constituent hydrocarbon solvents are primarily defined by distillation range and flash point. In addition, aliphatic hydrocarbon solvents are also defined by the amount of aromatic compounds they contain. The petroleum feedstocks used to make many aliphatic hydrocarbon solvents contains some aromatic hydrocarbons (normally less than 23%). For some aliphatic hydrocarbon solvents, such as regular mineral spirits or white spirits, these aromatic hydrocarbons are purposely retained in the final product to achieve or enhance certain solvency properties. The term dearomatized refers to those products that have undergone an additional step to remove or hydrogenate the aromatic compounds contained in the feedstock (e.g., dearomatized mineral spirits), resulting in an aliphatic hydrocarbon solvent with little or no aromatic content ( $\leq 2\%$ ). Some aliphatic hydrocarbon solvents, such as isoparaffins or n-paraffins, are produced through a polymerization process that involves the hydrogenation of an olefin (e.g. ethylene, propylene) oligimerization product. Petrochemicals produced in this manner are commonly referred to as synthetics because they are synthesized from smaller molecules rather than being derived from petroleum streams.

#### *Aromatic Hydrocarbon Solvents*

64. Aromatic hydrocarbons in the C9-C12 range are either one aromatic-ring structures with alkyl side chains (alkylbenzenes) or two aromatic-ring structures with alkyl side chains (alkylnaphthalenes). C9 aromatic hydrocarbon solvents contain isomers of trimethylbenzene, isomers of ethyltoluene, cumene (isopropylbenzene), n-propylbenzene and small amounts C8 and C10 aromatic hydrocarbons. Aliphatic

hydrocarbons of similar molecular weight may also be present in very small amounts, generally <1%. These products contain only traces of benzene (<10 ppm) and toluene (<100 ppm). Aromatic hydrocarbon solvents in the C10-C12 range contain isomers of alkylbenzenes and alkylnaphthalenes; some contain up to 10% naphthalene.

***Step 1: Identify structure-based category and its members.***

*Hydrocarbon Solvent Categories*

65. Nine categories were identified to address the different types of commercial hydrocarbon solvents in the C5-C20 range. These categories are listed below and each category is addressed in separate test plans.

- C5 Aliphatic Hydrocarbon Solvents
- C6 Aliphatic Hydrocarbon Solvents
- C7-C9 Aliphatic Hydrocarbon Solvents
- C9-C13 Aliphatic [ $\leq$ 2% Aromatics] Hydrocarbon Solvents
- C9-C13 Aliphatic [2-23% Aromatics] Hydrocarbon Solvents
- C14-C20 Aliphatic [ $\leq$ 2% Aromatics] Hydrocarbon Solvents
- C14-C20 Aliphatic [2-35% Aromatics] Hydrocarbon Solvents
- C9 Aromatics Hydrocarbon Solvents
- C10-C12 Aromatics Hydrocarbon Solvents

66. There are many different hydrocarbon solvent substances within the C5-C20 range being addressed. While there may be several potential ways to categorize and review these substances, the above categories appear to be the best way of organizing the substances based on physical-chemical properties (e.g. vapor pressure), commercial applications, uses, and toxicity. In developing these categories, it was intended that similar commercial products, with similar applications, be in the same category. Substances with similar hydrocarbon structures (e.g., aromatic) would be placed in the same category. Finally, special consideration was given to previously reviewed substances. For example, a separate category was developed for C9 Aromatic Hydrocarbon Solvents because of the U.S. EPA Toxic Substances Control Act (TSCA) C9 Test Rule on C9 aromatics (ethyltoluene and trimethylbenzene isomers).

*Underlying Hypothesis for the Formation of the Categories*

67. There are essentially two key underlying hypotheses for the formation of the hydrocarbon solvent categories. The first is that hydrocarbon solvents can be grouped around major product types (e.g., hexanes, VM&P Naphtha, Mineral Spirits/White Spirits, etc.). Because there are so many different commercial and trade names used for hydrocarbon solvents, it was decided that generic names (e.g., C7-C9 Aliphatic Hydrocarbon Solvents) would be the most appropriate for the OECD HPV Chemicals Programme. While commercial hydrocarbon solvent products are frequently defined by distillation range, it was felt that grouping hydrocarbon solvents by predominant carbon number range would be easier and more practical than grouping the hydrocarbon solvents by boiling range because of simpler nomenclature, easier identification, and selection of representative constituents for modelling. The second hypothesis for the formation of the categories is that aliphatic isomers within a particular carbon range, regardless of them being straight-chained, branched, or cyclic, and aromatic hydrocarbons within a particular carbon range have similar physicochemical, environmental fate, and toxicological properties. The two defining toxicological characteristics of hydrocarbon solvents are central nervous system depression and upper respiratory tract irritation observed at high airborne concentrations. The hypothesis that these two toxicity endpoints are related to and can be predicted by carbon chain length and aromatic content is

supported by reviews appearing in various standard toxicology texts, including Patty's Industrial Hygiene and Toxicology, Browning's Toxicology of Industrial Solvents, and Caserett and Doull's Toxicology. The single well-known exception is n-hexane, which produces a specific type of axonopathy resulting from metabolism to the gamma diketone 2,5-hexanedione. The specific structural requirement for the axonopathy induced by gamma diketones is also described in standard toxicology texts.

*Technical and Practical Issues in Forming the Categories*

68. The grouping of hydrocarbon solvents was proposed around hydrocarbon chemistry (e.g. isoparaffins, n-paraffins, alkylbenzenes) and major product types (C9-C13 Multi-constituent Hydrocarbon Solvents). There are a number of important technical issues when evaluating complex substances. One is that environmental fate and physicochemical data cannot be modelled for complex substances. To address this issue, representative constituents were identified for each category to cover the carbon range and hydrocarbon structures of the category. These constituents were then modelled for the SIDS environmental fate and physicochemical endpoints. Measured data on the commercial products is available for most of the physicochemical endpoints, so this is also provided. Generally the modelled constituent data and measured whole product showed good correlation, further supporting the categories. Major usage categories were also a practical consideration in forming the categories.

As an example, Table E.1 presents the proposed category for C7-C9 Aliphatic Hydrocarbon Solvents.

**Table E.1: Substances in the C7-C9 Aliphatic Hydrocarbon Solvents Category**

<b>Class/Dossier</b>	<b>CASRN</b>	<b>Chemical Name</b>
Normal Paraffins	142-82-5	Heptane
	111-65-9	Octane
	111-84-2	Nonane
Isoparaffins	70024-92-9	Alkanes, C7-8-iso-
	90622-56-3	Alkanes, C7-10-iso-
Multi-constituent	8032-32-4	Ligroine
C7-C9 Aliphatics	64741-63-5	Naphtha, (petroleum), light catalytic reformed
	64741-84-0	Naphtha, (petroleum), solvent-refined light
	64742-48-9	Naphtha, (petroleum), hydrotreated heavy
	64742-49-0	Naphtha, (petroleum), hydrotreated light
	64742-89-8	Solvent naphtha, (petroleum), light aliph.
	92045-53-9	Naphtha (petroleum), hydrodesulf. light, dearoma.
	426260-76-6	Heptane, branched, cyclic and linear

**Step 2: Gather published and unpublished data for each category member.**

**Step 3: Evaluate available data for adequacy**

**Step 4: Construct a matrix of data availability (SIDS endpoints vs. category members) and indicate in the cells of the matrix whether existing data are available.**

69. These three steps were essentially done almost in parallel once the categories were formed. Available data from company proprietary files, the peer-reviewed literature, and modelled data for environmental fate and physicochemical endpoints were collected, placed in a matrix and evaluated. Data were evaluated for study reliability in accordance with the OECD guidance. Only studies which met the reliability criteria of "1" (valid without restriction) or "2" (valid with restrictions) were included in this review. In a few cases, additional data for substances with the same CAS RN exist in the ECB (European Chemicals Bureau) IUCLID (International Uniform Chemical Information Dataset). These data were evaluated and included in the review only if it was determined that the substances were compositionally relevant and met the reliability criteria of "1" or "2." After these data evaluations were complete, the any necessary testing to fill data gaps was proposed. Preliminary recommended changes from EPA on the test plans included the proposed addition of chronic aquatic toxicity data for categories with a log Kow above 4.2. Table E.2 and E.3 provide the matrices for the C7-C9 Aliphatic Hydrocarbon Solvents Category.

**Table E.2 C7-C9 Aliphatic Hydrocarbon Solvents Category – Toxicity Endpoints Matrix**

<u>Endpoints</u>	<b>Multi-Constituent Substances (i-,n-,cy-)</b>	<b>Isoparaffins</b>	<b>Normal Paraffins</b>
<b>Acute</b>	1 <sup>1</sup> - Low	- Low	- Low
Assigned Value <sup>2</sup>	<b>Low</b>	<b>Low</b>	<b>Low</b>
<b>Repeat-Dose</b>	- No Target Organ	- No Target Organ	- CNS/No Target Organ
Assigned Value	<b>No target organ effects; CNS at high conc.</b>	<b>No target organ effects; CNS at high conc.</b>	<b>No target organ effects; CNS at high conc.</b>
<b>Genetic – in vitro</b>	-Negative (bac.) -Negative (mam.)	-Negative (bac.) -Negative (mam.)	-Negative (bac.) Read-Across (mam.)
Assigned Value	<b>Negative</b>	<b>Negative</b>	<b>Negative</b>
<b>Genetic – in vivo</b>	-Negative (new testing)	-Negative	Read-Across
Assigned Value	<b>Negative</b>	<b>Negative</b>	<b>Negative</b>
<b>Reproductive</b>	- No Repro Effects	Read-Across	Read-Across
Assigned Value	<b>No Repro Effects</b>	<b>No Repro Effects</b>	<b>? – Under Discussion</b>
<b>Developmental</b>	- No Develop Effects	- No Develop Effects	Read-Across
Assigned Value	<b>No Develop Effects</b>	<b>No Develop Effects</b>	<b>No Develop Effects (?)</b>

<sup>1</sup> - Study Available.  
<sup>2</sup> Assigned Value – Given available data, information, and construct of category, the value assigned to cells for which there are no data.

**Table E.3 C7-C9 Aliphatic Hydrocarbon Solvents Category – Aquatic Endpoints Matrix**

<b>Endpoints</b>	<b>Multi-Constituent Substances (i-, n-, cy-)</b>	<b>Isoparaffins</b>	<b>Normal Paraffins</b>
Acute Fish	D <sup>1</sup> – Moderate	Read-across	D - Moderate
Assigned Value <sup>2</sup>	Moderate	Moderate	Moderate
Acute Invertebrate	D - Moderate	Read-across	D - Moderate
Assigned Value	Moderate	Moderate	Moderate
Algae Toxicity	D - Moderate	Read-across	Read-across
Assigned Value	Moderate	Moderate	Moderate
Chronic Invertebrate	D (Test Underway)	Read-across	Read-across
Assigned Value			
<sup>1</sup> D - Study available <sup>2</sup> Assigned Value – Given available data, information, and construct of category, the value assigned to cells for which there are no data.			

*Use of Read-Across Data*

70. The categories were initially developed around commercial hydrocarbon solvents. The available data were then reviewed and the categories were evaluated based on this data; in some case the categories were reorganized. As these product categories are in sequence and data are available at a number of points across the spectrum of hydrocarbon solvents, use of read-across data was considered in some cases. Use of read across is limited to chemicals of very similar structure. For example, in the C7-C9 Aliphatic Hydrocarbon Solvents category data for C7-C9 normal paraffins, C7-C9 isoparaffins, and complex C7-C9 multi-constituent aliphatic substances (products containing normal, iso-, and cycloparaffins) were used to read across (either quantitatively or qualitatively) to cover all the substances in the category. Where data were not available and read-across could not be justified, additional testing was proposed.

***Step 5: Evaluate the category approach.***

71. Since most of the SIDS endpoint data for hydrocarbon solvents were already available, the verification of the two key category hypotheses generally occurred simultaneously to the assembly and evaluation of the data for each category. Generally the IHSC found that good correlation of the data suggesting that the proposed categories are appropriate. In one case, the initial assembly and evaluation of the data caused the reorganization of the categories. In this case, originally one category was considered for the C9-C13 Aliphatic Hydrocarbon Solvents. The toxicology endpoint data showed good correlation across this category; however, the products with higher aromatic content (up to 23%) in this category showed somewhat greater respiratory irritation and more water solubility due to the greater solubility of the aromatic fraction, resulting in correspondingly greater potential to cause aquatic toxicity at similar loading rates. To address this difference in irritation, water solubility and aquatic toxicity, it was decided to divide the category into two categories – one with essentially no aromatic content and one with products up to 23% aromatic content. Once the testing is complete, where planned, these additional data will be evaluated to determine whether the categories are valid or need to be reorganized.

***Step 6: Prepare category test plan***

72. Category test plans have been developed and were submitted to the Sponsor Country (U.S.) for evaluation and consideration prior to submission to OECD.

***Step 7: Conduct necessary testing***

73. Underway for those categories that have been evaluated by EPA.

***Step 8: Evaluate new and existing data for the category and make robust study summaries for new data***

74. To be completed upon completion of testing.

## Example F: INORGANIC NICKEL COMPOUNDS.

[Note: Inorganic nickel compounds have not been assessed by SIAM yet. The description below is based on the work of the Danish Environmental Protection Agency under the OECD SIDS and the EU existing chemicals programmes, as well as the discussions held at the OECD workshop on the development and use of chemical categories held in January 2004. The descriptions below might have to be revised once the compounds have undergone assessment at SIAM.]

### *Step 1: Identification of structure-based category and its members:*

75. The category is initially defined as “nickel and nickel compounds”. This description is a category already widely used in EU legislation. “Nickel and nickel compounds” includes over 300 compounds of very diverse chemical structure. Numerically, organic nickel compounds outnumber inorganic nickel compounds. The category includes a number of complex compounds, many of which are waste products. The wide diversity of chemical types suggests that whilst it is useful as a category in identifying compounds that contain nickel and may therefore potentially be a source of nickel release, it is not a useful category in the sense that it provides a basis for predicting effects that are similar to all members of the group.

76. A second type of grouping of nickel compounds is also used. This divides nickel compounds into five groups: Metallic nickel, oxidic nickel, sulphidic nickel, soluble nickel and nickel carbonyl. These groups reflect the nickel compounds seen during nickel refinery production, rather than the wider range of nickel chemicals on, or potentially on, the market. These different categories have been used in some countries as the basis for different Occupational Limit Values (OELs) based on differences in the types and potency of different mammalian toxicological effects.

77. There are a number of assumptions underlying any grouping of nickel compounds for estimating their biological properties. The main assumption is that it is the nickel ion that is responsible for the effects to be assessed. This is considered to be a reasonable assumption for the majority of the inorganic anions of nickel compounds and for some organic anions. This implies that in the case of inorganic metal salts, the hazard assessment is based on the known toxicity of the cation.

78. The basis of any grouping would therefore be the water solubility of the nickel salt. Two reports prepared for the Danish EPA by Lars Carlsen<sup>8</sup> have collected and assessed the available data for water solubility of inorganic nickel compounds (Carlsen, 2001a) and organic compounds (Carlsen, 2001b).

79. For inorganic nickel compounds, a grouping of inorganically based nickel species has been suggested. Nickel metal and nickel metal compounds can all be considered as insoluble. Nickel oxides and mixed metal oxides are also very similar in terms of their solubility. In the table below, a grouping of the nickel ligands with Group 13, 14, 15, 16 and 17 ligands is suggested. The term ‘insoluble’ means that the solubility of the species is less than  $10^{-4}$  mol/L, ‘slightly soluble’ covers the solubility range  $10^{-4}$  -  $10^{-2}$  mol/L, ‘soluble’ the range  $10^{-2}$  -  $5 \cdot 10^{-1}$  mol/L and ‘very soluble’ refers to solubility above  $5 \cdot 10^{-1}$  mol/L.

80. It should be noted that the group of “insoluble” compounds, with solubility  $< 10^{-4}$  mol/L, may nevertheless have a solubility in excess of 1 mg/L, depending on the actual solubility and the molecular

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<sup>8</sup> Carlsen L (2001a): Aqueous Solubilities and Complex Stabilities of Ni(II) Species. Part I: Inorganic Ligands. Draft report to the Danish EPA.

Carlsen L (2001b): Aqueous Solubilities and Complex Stabilities of Ni(II) Species. Part II: Organic Ligands. Draft report to the Danish EPA.

weight. Hence substances conventionally thought of as “insoluble” by chemists or toxicologists may still be sufficiently soluble to be regarded as such in evaluating effects on the aquatic environment.

*Grouping of nickel species based on inorganic ligands in water (from Carlsen, 2001a).*

	Group 13	Group 14	Group 15	Group 16	Group 17	Misc.
Insoluble	Ni <sub>x</sub> B	Ni <sub>x</sub> Si	Ni <sub>x</sub> P <sub>y</sub> Ni <sub>x</sub> As Ni <sub>x</sub> Sb <sub>y</sub> Ni <sub>2</sub> P <sub>2</sub> O <sub>7</sub> Ni <sub>3</sub> (AsO <sub>3</sub> ) <sub>2</sub> Ni <sub>3</sub> (AsO <sub>4</sub> ) <sub>2</sub> Ni(AsO <sub>3</sub> ) <sub>2</sub>	Ni <sub>x</sub> S <sub>y</sub> Ni <sub>x</sub> Se Ni <sub>x</sub> Te		Ni <sub>2</sub> Fe(CN) <sub>6</sub>
Slightly soluble		Ni(CO) <sub>4</sub> Ni(CN) <sub>2</sub> NiCO <sub>3</sub> Ni(HCO <sub>3</sub> ) <sub>2</sub>	Ni <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> Ni[NiP <sub>2</sub> O <sub>7</sub> ]	NiSO <sub>3</sub> <sup>a</sup> NiSeO <sub>3</sub>	Ni(IO <sub>3</sub> ) <sub>2</sub>	Ni <sub>2</sub> Fe(CN) <sub>5</sub> NO
Soluble				NiK <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub>	NiF <sub>2</sub>	
Very soluble	NiB <sub>6</sub> O <sub>10</sub> Ni(BF <sub>4</sub> ) <sub>2</sub>	Ni(SCN) <sub>2</sub> NiSiF <sub>6</sub>	Ni(NO <sub>3</sub> ) <sub>2</sub> Ni(H <sub>2</sub> PO <sub>2</sub> ) <sub>2</sub>	NiSO <sub>4</sub> Ni(SO <sub>3</sub> NH <sub>2</sub> ) <sub>2</sub> <sup>a</sup> NiSeO <sub>4</sub>	NiCl <sub>2</sub> Ni(ClO <sub>3</sub> ) <sub>2</sub> Ni(ClO <sub>4</sub> ) <sub>2</sub> NiBr <sub>2</sub> Ni(BrO <sub>3</sub> ) <sub>2</sub> NiI <sub>2</sub>	

81. No comparable grouping of organic ligands has yet been carried out (Carlsen, 2001b). In contrast to the inorganic nickel compounds it is not obvious how to group the organically based species based on solubilities alone. Aqueous solubilities are not unexpectedly seen to decrease with increasing molecular weight and increasing carbon content of the ligand. On the other hand, the introduction of hydrophilic and/or polar functional groups, such as OH, C=O, COO<sup>-</sup>, NH, SH and SO<sub>3</sub><sup>-</sup> cause increased solubilities. Further it should be emphasized that the solubility of the complexes cannot immediately be related to the solubility of the single ligands. Hence, it seems more appropriate to group organically based nickel complexes based on the stability of the complexes. As a first attempt, grouping the individual complexes based on the nature of the ligand appears as an obvious choice, even though significant variations in stability may prevail within the single groups. However, a number of nickel salts of simple organic acids can be considered to behave in a similar way to inorganic salts with a similar solubility.

***Step 2: Gather published and unpublished data for each category member.***

82. There is a vast database on the human health effects of nickel compounds. A search in Toxline gave 2538 hits for nickel and toxicity, 5077 hits for nickel and effects and about 16000 hits for nickel and sensitisation. However, the data available for any individual nickel compounds can vary considerably. The two compounds for which there is data that covers most endpoints are the two soluble compounds, nickel chloride and nickel sulphate. Much of the database relating to nickel metal is linked to

sensitisation. On the other hand, there is virtually no data at all for most nickel compounds. In particular, data on the organic nickel compounds is extremely limited.

***Step 3: Evaluate data for accuracy.***

83. Much of these human health data have been reviewed in good quality reviews including UK HSE (1987)<sup>9</sup>, IARC (1990)<sup>10</sup>, IPCS (1991, 1996)<sup>11</sup>, US ATSDR (1997)<sup>12</sup> and a Nordic Expert Group (Aitio, 1995)<sup>13</sup>. NiPERA in collaboration with Eurométaux have also produced a criteria document for nickel and nickel compounds for the European Commission (NiPERA 1996)<sup>14</sup>. Toxicology Excellence for Risk Assessment (TERA) has prepared a toxicological review of soluble nickel salts for Metal Finishing Association of Southern California Inc., US-EPA and Health Canada (TERA 1999)<sup>15</sup>.

84. In depth reviews of metallic nickel, nickel sulphate, nickel chloride, nickel nitrate and nickel (hydroxy)carbonate have been prepared by the Danish EPA.

***Step 4: Construct a matrix of data availability.***

85. A matrix of available data included in the draft risk assessment reports prepared under the OECD SIDS and the EU existing chemicals programmes is shown below for nickel metal, nickel sulphate, nickel chloride, nickel nitrate and nickel carbonate<sup>16</sup>.

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<sup>9</sup> Toxicity Review 19. The toxicity of nickel and its organic compounds. Fairhurst & Illing. London. HMSO. ISBN 0 11 883961 6

<sup>10</sup> IARC Monographs on the evaluation of carcinogenic risks to humans, Volume 49, Chromium, nickel and welding. IARC, Lyon, France, 1990. pp. 257-446.

<sup>11</sup> Environmental Health Criteria 108: Nickel. World Health Organisation, Geneva. 383 p.; IPCS (1996): Guidelines for drinking water quality. Volume 2. Health criteria and other supporting information. World Health Organisation, Geneva, 1996 p. 308-313.

<sup>12</sup> Toxicological Profile for Nickel. September 1997. US Department of Health and Human Services, Public Health Service.

<sup>13</sup> Nickel and nickel compounds. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. Arbete och Hälsa vetenskaplig skriftserie 1995:26, no 119. Solna: Arbetslivsinstitutet, 1995: pp. 1-61.

<sup>14</sup> Occupational exposure limits: Criteria Document for nickel and nickel compounds. Volume I: Summary, Conclusions and Recommendations; Volume II: Assessment of Occupational Exposures; Volume III: Health Assessment of various species of Nickel. Prepared by NiPERA in collaboration with Eurométaux for the European Commission, Directorate General V. Public health and Safety at Work Directorate. Batiment Jean Monnet, Plateau du Kirchberg. L-2920 Luxembourg.

<sup>15</sup> Toxicological review of soluble nickel salts. Prepared for: Metal Finishing Association of Southern California, Inc., US Environmental Protection Agency and Health Canada. Prepared by Toxicology Excellence for Risk Assessment (TERA) under subcontract in part with Science Applications International Corporation (SAIC). EPA Contract #68-C7-0011. March 1999.

<sup>16</sup> The compound reported as a HPV chemical to IUCLID was nickel carbonate (CAS No. 3333-67-3). In the course of subsequent discussions with the Industry, it became clear that the marketed product was in fact a nickel hydroxycarbonate. For administrative purposes, the commercial product is considered to be the 1:2 hydroxycarbonate, [carbonato(2-)] tetrahydroxytrinickel, (CAS No. 12607-70-4) which is also included in the TSCA Inventory. As it is not always clear from the study reports which precise carbonate has been tested, the results are shown as "nickel carbonate".

86. Data is also available for some other nickel compounds in the reviews quoted above and from the data included in IUCLID. As no substance-specific reviews have been carried out on these substances, the data available for each substance is regarded as indicative only. Further work is needed to refine this matrix.

87. In addition data is available on the EU provisional categorization supplied by the producer/importer from IUCLID is available for a number of other nickel compounds, including a number of organic compounds and complex waste products such as slimes and sludges. It is not clear whether these have been based on experimental evidence or on assumptions about the properties of the compounds (i.e. application of a group approach).

88. The main nickel compounds studied are those directly associated with the (refinery) production of metallic nickel, and nickel alloys. Some of the intermediate products (nickel matte, ferro-nickel) in this production process do not appear to have been studied. The data on nickel compounds not directly associated with these processes appears to be very limited. The information on “downstream” nickel compounds, and in particular, the organic nickel compounds, is limited. The information available from IUCLID is difficult to interpret as there is little or no experimental data reported for these substances.

Matrix of data availability on selected nickel compounds.								
Nickel compound	Environmental fate	Ecological effects*			Human Health effects **			
		Fish acute	Daphnid acute	Daphnid chronic	Acute	Repeated dose	Mutagenicity	Developmental
<b>nickel metal***</b>	dissolution protocol	-	-	-	√	√	(√)	-
<b>nickel oxide</b>	transformation test	√	√	-	√	√	√	-
<b>nickel sulfide / subsulfide</b>	screening test	-	√	-	√	√	√	-
<b>nickel dihydroxide</b>	screening test	-	√	-	√	-	(√)	-
<b>“nickel carbonate”</b>	dissolution protocol.	-	-	-	√	(-)	(√)	-
<b>nickel acetate</b>	soluble	-	-	√	√	-	√	-
<b>nickel sulphate</b>	soluble	√	√	√	√	√	√	√
<b>nickel chloride</b>	soluble	√	√	√	√	√	√	√
<b>nickel nitrate</b>	soluble	√	√	√	√	-	√	-
<b>nickel carbonyl</b>	soluble	-	√	-	√ ****	-	-	-

Key: “√” denotes data available for the substance/endpoint. There may not necessarily at present be agreement on the interpretation of this data. “(√)” indicates that there is some data, but that additional data may be needed. “(-)” indicates only very limited data from which no conclusions can be drawn. “-” denotes no data available.  
 Shaded areas show six possible subgroups (the five subgroups shown in step 1 and sparingly soluble nickel hydroxide and carbonate).  
 \*: data concerning other endpoints and species are available and are being considered.  
 \*\*: data is also available for sensitisation and carcinogenicity  
 \*\*\*: nickel metal powder (INCO123) and nickel granules have been tested. Only the powder has been tested in the 28 d dissolution test however  
 \*\*\*\*: data available for inhalational exposure. Data for other nickel compounds is oral data only.

89. The matrix shown above includes the main SIDS endpoints. However, major concerns with nickel and nickel compounds are related to sensitisation and carcinogenicity, endpoints not included in SIDS. Evaluation of these endpoints is important in the evaluation of this particular group of substances.

***Step 5: Perform an internal assessment of the category.***

90. The subgroup for which most data is available are the soluble nickel salts. The available data suggests that “read-across” within this group is justified. The available data also suggest that the effects of the different nickel compounds are related to water solubility, although different endpoints may behave differently.

91. In applying the aquatic hazard classification rules for nickel compounds, soluble and slightly soluble compounds can be distinguished. For the soluble compounds, no T/D protocol is required. For slightly soluble compounds, the use of T/D protocol, i.e. solubility dependency of pH at environmentally realistic pHs is used.

92. Acute oral toxicity decreases with decreasing water solubility and is of concern for soluble and slightly soluble compounds. Inhalational repeated dose toxicity on the other hand is shown by both soluble and insoluble nickel compounds. There is also evidence of *in vivo* mutagenicity for both soluble and insoluble compounds, although the evidence for insoluble compounds is much less than for soluble compounds. Whilst there is an effect on developmental reproductive toxicity for the soluble nickel compounds, there is little data on which to evaluate this effect in slightly soluble or insoluble compounds.

93. Whilst the environmental effects of nickel carbonyl appear to be consistent with the results expected from its water solubility, its effects on human health are not like any of the other nickel compounds studied. The valence state of nickel in this compound is Ni(0) rather than Ni(II) in most of the other compounds studied.

94. The available data for metallic nickel for key endpoints such as carcinogenicity is not adequate to assess the effects of the metal.

***Step 6: Prepare category test plan.***

***Step 7: Conduct necessary testing.***

***Step 8: Perform an external assessment of the category.***

95. Additional testing is currently underway to evaluate certain aspects of the carcinogenicity of nickel compounds. Metallic nickel is being tested following inhalational administration and nickel sulphate following oral administration.

96. Industry has initiated a research programme concerning the influence of abiotic factors on the (chronic) ecotoxicological effects of nickel using the BLM theory<sup>17</sup>.

97. There are no plans at the present time for specific testing aimed at providing data on this category as a whole.

***Step 9: Fill data gaps by read-across, extrapolation, interpolation etc.***

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<sup>17</sup> Biotic Ligand Model

98. The use of read-across for specific endpoints within the limited group of substances for which data is available is clearly acceptable. This being the case, it seems reasonable to consider to what extent the same approach can be applied to the much larger group of “nickel and nickel compounds”.

99. In cases where there are clear similarities to the compounds considered above, the use of read-across to evaluate the hazards of these compounds would seem justified. For example, soluble nickel(II) salts would be expected to show the same effects as the other soluble salts evaluated on the basis of their measured data.

## ANNEX 2

### DEFINITIONS

1. Structure-Activity Relationships (SARs) and Quantitative Structure-Activity Relationships (QSARs), collectively referred to as (Q)SARs, are theoretical models that relate chemical structure to a physicochemical property, environmental fate parameter, toxicological (human health) or ecotoxicological (environmental species) effect.

2. A SAR is a qualitative association between a chemical substructure (called a structural alert or pharmacophore) and an effect or biological activity. The association can be positive (if the chemical substructure is associated with the *presence* of the effect/activity) or negative (if the chemical substructure is associated with the *absence* of the effect/activity). A structural alert is a two-dimensional fragment, whereas a pharmacophore is a three-dimensional arrangement of key molecular features.

3. Structural alerts are hypotheses that are generally based on the observation that, within a set of chemical structures, the proportion of chemicals containing the fragment and exhibiting the presence (or absence) of the effect/activity is greater than the proportion of chemicals lacking the fragment and exhibiting absence (or presence) of the effect/activity. An example would be the assumption that a new chemical entity containing an aromatic amine grouping is likely to exhibit skin sensitising effects (Cronin et al, 2003).

4. Pharmacophores are hypotheses that are generally based on molecular modeling studies in which similar chemicals are compared in terms of their three-dimensional shape and distribution of charge, and commonalities are observed in the three-dimensional arrangement of key features between chemicals exhibiting the presence (or absence) of a certain/activity. An example would be a pharmacophore for predicting the estrogenicity of steroidal molecules (Fang et al, 2001).

5. A QSAR is a quantitative (mathematical) relationship between a numerical measure of chemical structure, or a physicochemical property, and an effect/activity. QSARs often take the form of regression equations, and can make predictions of effects/activities that are either on a continuous scale or on a categorical scale. Thus, in the term “QSAR”, the qualifier “quantitative” refers to the nature of the relationship, not the nature of the endpoint being predicted. An example of a QSAR would be the prediction of acute toxicity to an invertebrate species (*Tetrahymena pyriformis*) by means of a regression equation with the partitioning behaviour (log P value) of the chemical as a descriptor (Schultz et al, 2002).

6. A Quantitative Activity-Activity Relationship (QAAR) is a mathematical relationship between two types of biological activity. In general, a QAAR expresses the correlation between a biological endpoint in one species and a “similar” endpoint in another species. A QAAR can be used to extrapolate from an invertebrate species to a vertebrate species, thereby reducing the need for animal experimentation. An example would be the prediction of acute toxicity to the guppy fish by using data on acute toxicity to the ciliated protozoan *Tetrahymena pyriformis* (Seward et al., 2002).

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