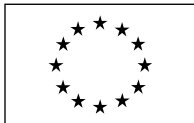# Chemical Categories and Read Across

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**ABSTRACT**

This report aims to outline and summarise some of the practical experiences from the major Regulatory agencies as well as the guidance so far developed by the OECD. Definitions for some of the common terms such as read across and chemical categories are provided. Recommendations for further work are highlighted.  Regulatory use of read across/chemical category is still quite limited. A clear need is the development of practical guidance to promote greater uptake of these types of these approaches.

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**BASIC CONCEPTS**

The underlying premise underpinning all Structure Activity relationships (SAR) is the expectation that structurally similar chemicals will have similar physical attributes and biological effects.

**Definitions**

A <u>Read-across/analogue approach</u> typically involves using data/information on one chemical structure and making some assessment about the relevance of that information for a second chemical structure. It is the process by which one or more properties of a given chemical are inferred by comparison of that chemical with a chemical(s) of similar molecular structure(s) and physicochemical properties, for which the properties of interest are known. This approach can be used to assess physicochemical properties, toxicity, environmental fate and eco-toxicity.

The read-across can be performed qualitatively or quantitatively:
<u>Qualitative read-across</u> can be regarded as the application of SAR. The process involves:
a) the identification of a chemical substructure that is common to the two substances (which are therefore analogues); and
b) the assumption that the presence (or absence) of a property/activity for a substance can be inferred from the presence (or absence) of the same property/activity for an analogous substance.
This assumption implies that analogues behave qualitatively similarly, and is usually the result of an expert judgement evaluation.
<u>Quantitative read-across</u> involves the identification of a chemical substructure that is common to the two substances (which are therefore analogues), and the assumption that the *known* value of a property for one substance can be used to estimate the *unknown* value of the same property for another substance. This assumption implies that the potency of an effect shared by different analogous substances is similar, and is also usually the result of an expert judgement evaluation.

<u>SAR and QSAR</u>: A (Q)SAR consists of a relationship between the chemical structure, or physical-chemical representations thereof and the outcome in a test for an endpoint (biological or other physicochemical. property). They can be divided into two major types, <u>QSARs and SARs</u>.

<u>SARs</u> are qualitative relationships in the form of structural alerts that incorporate molecular substructures or fragments related to the presence or absence of activity.
They normally involves use data from a training set of many chemicals and their structures, the development of rules based on "expert judgement" and then applying the rules to another chemical structure.

<u>QSARs</u> are quantitative models yielding a continuous or categorical result. The most common techniques for developing QSARs are regression analysis, neural nets and classification methods. Examples of regression analysis include ordinary least squares and partial least squares. Examples of classification methods are discriminant analysis, decision trees and distance based methods of similarity analysis. QSARs normally involve the development of a mathematical (statistical) based equation, based on the data from a training set of many chemicals and their structures/properties, which may then be applied to another chemical structure.

The major difference between these approaches and those described for analogue/read across, are that a (Q)SAR is often based on larger numbers of chemicals and tends to be more formalised in its description.

A <u>chemical category</u> is a group of chemicals whose physicochemical and toxicological (including ecotoxicological) 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 properties, environmental fate and environmental effects, and/or human health effects. The similarities may be based on the following:

- a common functional group (e.g., aldehyde, epoxide, ester, etc.) related to specific activity; or

- the likelihood of common precursors and/or breakdown products, via physical or biological processes, which result in structurally similar chemicals (e.g., the "family approach" of examining related chemicals such as acid/ester/salt); and

- an incremental and constant change across the category (e.g., the methylene group difference between adjacent members of the alpha-olefins).

Within a category different members may be selected for the endpoint desired. If the available test results show that the chemicals in a category behave in a similar or predictable manner, then interpolation and/or extrapolation may be used to assess the chemicals instead of conducting additional testing.


**Evaluation of Analogues/Read-across and Chemical Category approaches**

<u>Analogues/read-across</u>

Since a read across may involve as little as two chemicals, only the data for the first chemical needs to be known together with the detailed reasoning behind the comparison.


<u>Chemical categories</u>

The robustness of a chemical category could be evaluated in a similar way to that of QSARs. The features that relate the category members is assessed together with an assessment of the scope of the category. In principle, a representative set of chemicals within the scope could be identified and tested in the same way as would be done for an external validation of a QSAR.

**Use IN EU**

<u>UK</u>

UK experience that is currently known about in the use of read across comes principally from the **Health and Safety Executive** (HSE) and the UK Environmental Agency.

The HSE follows a series of needs and principles that were laid out by Hanway and Evans in 2000.

There are a number of steps which are followed on a case-by-case basis. Firstly a chemical is evaluated on the basis of its structural similarity e.g. Is there an absence or presence of specific functional groups that might modify the likely activity/toxicity expressed?

Hence the purity and impurity profile is considered to judge whether this might have an impact on the toxicity profile. The physicochemical properties are compared since properties such Log P, aqueous solubility may provide insights to the likely absorption characteristics of a chemical. The likely toxicokinetics are evaluated to consider how stable the chemical is and whether it will metabolise, decompose or hydrolyse in some manner. DEREK for Windows, the knowledge based expert system is used to identify structural alerts if any. All available toxicological information is collated and assessed.

These steps are considered in turn and form the basis of any read across argument. Typically some toxicity testing is requested to confirm the validity of the read across; an acute oral toxicity study and an Ames test are conducted. If the results of these studies are different, then further testing is conducted.

Several conclusions have resulted from this approach: a) acute oral toxicity testing and Ames testing has been effective in underpinning a read across argument; b) for regulatory purposes it is usually easer to read across positive data; c) two extremes in a series are sufficient to define the domain; and d) new tests should not be conducted to remove unwanted classifications.

*Suggestions for the further development of guidance*

This approach has been demonstrated to be useful in reading across a number of chemicals under NONs on a case-by-case basis. It could be potentially extended to help formulate larger groupings of chemicals as initial categories.

However what is not apparent from the information available are the practical steps of how a read across analog is selected or whether mechanistic considerations are accounted for in this selection.

Further information on how analogues are selected would be helpful to understand the overall approach better and what modifications may be required in order for EU guidance to be drafted for REACH.

**References:**
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Potts RO, Guy RH. (1992). Predicting skin permeability. *Pharm Res*. 9(5), 663-669.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ.(2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 46(1-3), 3-26.

The **UK Environmental Agency** have a similar approach to read across as the HSE though appropriate ecotoxicity tests such as acute toxicity to Daphnia form the basis of establishing the validity of their read acrosses.The approach is also a step-by-step one. Structural similarity is assessed including an evaluation of whether there are any additional functional groups (or absence of groups) that might modify the toxicity. Then the purity and impurity profile is assessed. An evaluation of the physicochemical properties is made along with an assessment of how these properties may drive

ecotoxicity. QSARs for determining the likely toxicity of analogues are used. Basic toxicity tests such as acute toxicity to Daphnia are conducted to confirm the validity of a read across. In contrast to the approach by HSE, additional testing may be conducted to remove unwanted classifications.

### *Suggestions for the further development of guidance*

The evaluation of physicochemical properties is highlighted as a useful means to evaluate similarity as these types of parameters often drive the ecotoxicity response – this reflects mechanistic thinking that is employed in the read across. Examples mentioned included water solubility and Log P the octanol/water partition coefficient. This approach has been demonstrated to be very useful in reading across a number of chemicals under NONs on a case by case basis. The basis of this read across approach appears robust as mechanistic principles are implicitly embedded though further information that details how chemicals are selected for read across or what similarity approaches are used would be useful.

**OECD GUIDANCE**

Guidance on the formation and use for chemical categories for fulfilling data requirements has been published by the OECD as part of the OECD Manual for Investigation for HPV Chemicals (OECD, 2004). This guidance is used among others for fulfilling the data requirements within the OECD HPV Chemicals Programme. The same guidance document is published by the US EPA for use within the US HPV Challenge Programme. The OECD guidance document was revised during 2004 and 2005.

The revised guidance document addresses the following issues:
- definitions and explanations of the chemical category concept
- general approach for developing categories
- differences in grouping for different endpoints
- use of QSARs for the development of a category
- guidance on different types of categories (i.e. chain-length, metabolic pathways, isomers and their mixtures, complex substances, metal and metal compounds)

The guidance document also provides a number of examples of categories that have been adopted within the OECD HPV Chemicals Programme or are currently under preparation:
- Alpha-olefins - discrete chemicals with an incremental and constant change across the category
- Linear alkyl benzenes - family of mixtures
- Brominated diphenyl ethers - family of congeners
- Butenes – family of isomers and their mixtures
- Hydrocarbon solvents – family of complex mixtures
- Inorganic nickel compounds

*General Suggestions for the further development of guidance*

The main limitations of the revised guidance document (July 2005) are:
- Absence of systematic approach for category identification. The formation of chemical categories has been largely influenced by practical considerations such as the substances produced by the member of the industry consortium preparing the assessment. Although tools to systematically identify analogues within a large list of substances (e.g. OECD HPV List or EINECS) are being developed, they are not being used systematically.
- Absence of quantitative validation criteria. While the guidance document gives qualitative guidance for deciding whether a category is robust (e.g. establishment of a pattern for the results for a given endpoint across the members of the category), the decision remains largely an expert judgement.
- Tentative guidance on quantitative filling of data gaps. The guidance document provides only tentative guidance on how data gaps could be filled quantitatively by read-across, extrapolation or interpolation.
- Limited guidance for complex substances. Only tentative guidance with limited examples is provided for complex multi-component substances (mixtures) and metal compounds, in spite of the fact that these are types of compounds where this approach is currently used.

**Guidance on the Development and Use of Chemical Categories in the HPV Chemicals Programme**

Guidance on the formation and use for chemical categories for fulfilling data requirements has been published by the OECD as part of the OECD Manual for Investigation for HPV Chemicals (OECD,

2004). This guidance is used among others for fulfilling the data requirements within the OECD HPV Chemicals Programme where there are more than 5000 chemical substances on the OECD List.


**Definitions**


A <u>chemical category</u> 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 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 series 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).

The <u>applicability domain</u> of a chemical category defines the physicochemical property space within which the chemical category is considered to be valid. The applicability domain is a concept borrowed from the QSAR field. In the context of a chemical category, it can be considered to define 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). 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.

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.

<u>Read-across</u> 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 exists.

> <u>Qualitative read-across</u> 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.

> <u>Quantitative read-across</u> 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 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 rather than a more formal (mathematical) analysis.

<u>SARs</u> can also be applied by using data that are external to the chemical category. The process involves: a) the identification of a chemical substructure that is shared by a category member and by one or more chemicals (analogues) that do not belong to the category; 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 analogous chemicals (which are outside the category). Data from chemicals which are external to the category should not be used selectively for only those endpoints which support the category, unless justified on a scientific basis.

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) from a numerical measure of chemical structure (or physicochemical property).

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 In general, interpolation between category members is preferred to 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 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**
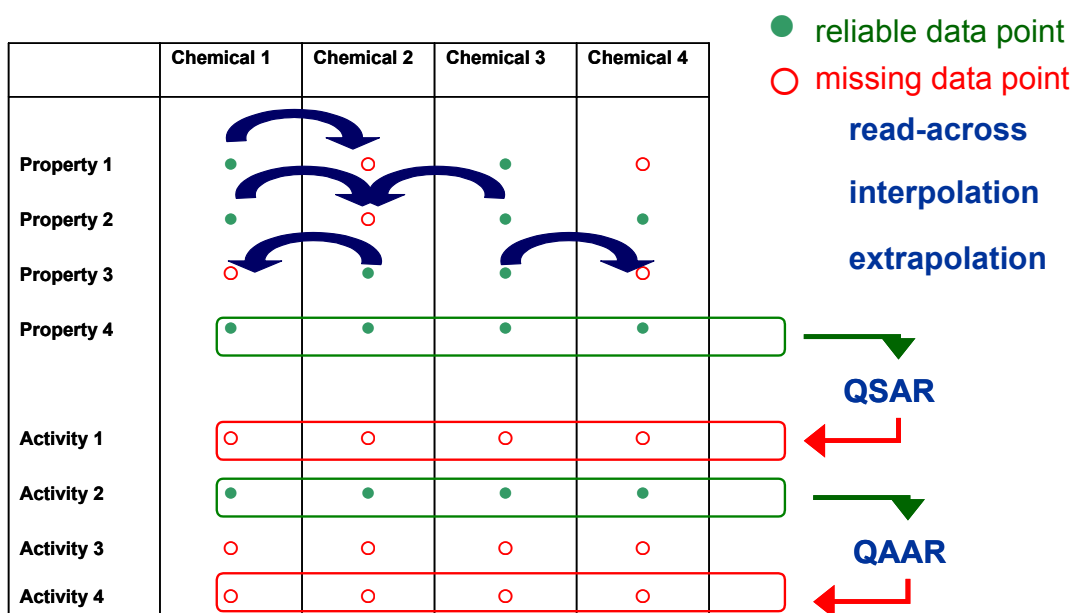


Within a category different members can be selected to demonstrate the pattern or trend of interest - i.e., those selected for a category approach for environmental effects endpoints may not be suitable for assessing human health effect endpoints. Furthermore, within a category, correlations might be established for different members of the same category depending on the property. 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.

**General Approach for Developing Categories**

HPV chemical category development is a step by step process which is described below.

- **Step 1: Define the category**

  A category can be defined in a variety of ways. Traditionally, category definitions have referred to chemical classes (e.g. epoxides), but a category definition could also refer to a group of chemicals related by a particular property (e.g. surfactants) or mechanism of action (e.g. non-polar narcotics).

  Some categories (e.g. propyl series) have been defined in terms of a common metabolic pathway. An alternative way of treating the members of such categories would be to locate them in categories defined on a different basis (e.g. by chemical class), and to define the chemical and/or biochemical reactions that relate one category to another. In other words, it is debatable whether chemicals related by a kinetic process should be placed in a single category or in multiple categories, since the kinetic data could either confirm or undermine a category defined on a different basis.

  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 define the ranges of values within which reliable estimations can be made for category members

  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.

  In identifying a category, it is important that all potential category members are described as comprehensively as possible. This is especially important where the group of chemicals is related by a particular property (e.g. surfactants) or mechanism of action (e.g. non-polar narcotics).

  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 whether or not the substances are marketed commercially.

  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).

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

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

  Construct a matrix of data availability 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.** 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 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 substantial data, i.e., adequate data for a given 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 endpoint. However, an alternative category proposal may be developed (go back to Step 1).
  - If there are substantial and adequate data that correlate well, the category may be appropriate and a category test plan proposal should be prepared (Step 6).
  - If substantial and adequate data do not exist, but the structure-based category is valid for one or more 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.

- **Step 6:** **Prepare category test plan.**
  Category test plans should include a category definition, rationale, and matrix of data availability and be accompanied by 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 allows 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. Assuming the endpoints are rows in the matrix, each row must have data in at least one cell. 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 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.

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

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

  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 that were not used to develop the model.

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

  The way to fill data gaps through the category approach is specific to each category. No definitive guidance can be provided for the moment.
  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.

*Specific Suggestions for the further development of guidance*

The OECD guidance is an excellent starting point in describing the principles and approaches of chemical categories. It does miss the specific technical input that would help an end user in starting to formulate a category i.e. what tools/resources are available that could facilitate that selection. The category description is largely limited to chemical classes, similar functional groups etc. Ideally this would be extended to a two-tiered approach to categories. On one simple level the category would be based on different measures of structural similarity (fingerprints, descriptors, pharmacophores etc)

with corresponding approaches or means by which a set of structures (potential analogues) could be identified. This first tier would describe the initial grouping. A second tier would focus in the parameters/descriptors driving a particular (eco)toxicity response i.e. mechanistically based groupings.

The guidance does not provide any assistance in terms of resources for extracting data. There are some resources that permit the search and retrieval of information for chemicals on the basis of structure or substructure and these could be described to highlight the "how".

Some guidance on what is meant by "adequate" as this is a subjective term and open to many different interpretations. Perhaps a systematic/robust means of accounting for "adequacy" would be in terms of the uncertainty of the data available and the impact of that on the risk assessment decision under consideration.

**Use of QSARs for the Development of a Category**

Greater confidence and further demonstration of the category approach may be gained through applying appropriate QSAR models on all category members for a given 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.

The output of QSAR modelling is particularly valuable in hypothesis generation and testing for step 1, "identifying the structure based category and its members". In particular, 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. It is also conserving of resources since it avoids consideration at early stage of potentially large volumes of data on, for example, multiple endpoints for human health. It also permits hypothesis testing of several possible combinations and permutations for category definition.

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.

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.

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 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.

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.

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 predictive output informs additionally to the data (i.e., 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).

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.

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.

For step 8 ("Perform an external assessment of the category"), 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.


**Guidance on different types of categories**


Chain length

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 where each category member differs by a –CH$_2$- unit and the ethylene glycols where there is an incremental increase in the CH$_2$CH$_2$O group.

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. Careful thought should be given to selecting the boundaries of a chain length category. The cut-off points described above may provide useful boundaries.

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 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

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 insistences, 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 predominate 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.

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.

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 born 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.

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.

An additional limitation of metabolic categories approach is that metabolism and toxicokinetics experiments have to be conducted with the parent compound.

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.

The following specific issues should be taken into account when developing a metabolic pathway category.

- at 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.

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.

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).

Chemical mixtures

Categories can sometimes apply to series of chemical reaction products or chemical mixtures 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.

Isomers and their mixtures

Isomers are chemicals that have identical molecular formula 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.
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

- positional isomers, for example hydrocarbon chains with a functional group that varies in position along the chain

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 $C_4H_{10}O$ as a molecular formula are 1-butanal and 2-butanone. Each of these isomers contain a carbonyl group (C=O), but are representative of two different chemical families, aldehydes and ketones. 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.
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.

There can be instances within a category of isomers, specifically as related to structural isomers, when read-across for an endpoint is not appropriate.

Complex substances

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[1] 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.

Category approaches for complex substances may vary, though generally the approach will be related to how the substances are manufactured, defined and used. This approach is practical and has the benefit of making sure that similar commercial products are grouped together in the same category.
- 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:
  - Components (what is present in the 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

The concept of chemical categories has traditionally been widely used for inorganic substances. However, there is not much experience is available to date of a systematic use of this approach. 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 anion is assumed to be largely irrelevant in producing the effects to be assessed. 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.

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.

- 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
- 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. .

Similar considerations would also apply to salts of anions where there are concerns for toxicity (e.g. cyanides, oxalates).

**USE BY US EPA**

**Chemical Categories**

The chemical categories are a good reflection of what is "knowledge within the EPA". The categories are typically biased towards environmental endpoints with limited justication for how the category or its members were selected. Human expert judgement is used to devise these categories. Prior to 1987, nearly 20% of Pre Manufacture Notifications (PMNs) submitted underwent a detailed review ("standard review") by EPA, a highly resource-intensive effort that lasted most of the mandated 90-day PMN review period. In 1987, after several years of experience in the review of PMNs, EPA's Office of Toxic Substances (now the Office of Pollution Prevention and Toxics) had enough accumulated experience to group PMN chemicals with shared chemical and toxicological properties into categories, enabling both PMN submitters and EPA reviewers to benefit from the accumulated data and past decisional precedents allowing reviews to be facilitated. Candidate categories for the New Chemicals review process are proposed by New Chemicals Program staff, based on experience reviewing PMNs on similar substances. At proposal, the database supporting the category is scrutinized for quality and for general applicability to other potential members of the category. Based on this analysis, a category statement is prepared describing the molecular structure. Boundary conditions such as molecular weight, equivalent weight, the log of the octanol/water partition coefficient (log P), or water solubility, that would determine inclusion in (or exclusion from) a category, and standard hazard and fate tests to address concerns for the category are all considered. The categories may not be made up of the most hazardous chemicals, but rather include chemicals for which sufficient history has been accumulated so that hazard concerns and testing recommendations vary little from chemical to chemical within the category. The categories are not intended to be a comprehensive list of all substances. The 64 categories are listed in the following table. A couple of example category descriptions are presented underneath.

| Acid Chlorides | Anhydrides, Carboxylic Acid | Cationic (quaternary ammonium) surfactants | Hindered Amines | Peroxides | Respirable, Poorly Soluble Particulates |
|---|---|---|---|---|---|
| Acid Dyes and Amphoteric Dyes | Anilines | Cobalt | Imides | Persistent, Bioaccumulative, and Toxic (PBT) Chemicals | Rosin |
| Acrylamides | Dianilines | Diazoniums | Diisocyanates | Phenolphthaleins | Stilbene, derivatives of 4,4-bis(triazin-2-ylamino)- |
| Acrylates/Methacrylates | Anionic Surfactants | Dichlorobenzidine-based Pigments | ß-Naphthylamines, Sulfonated | Phenols | Thiols |
| Aldehydes | Azides | Dithiocarbamates | Lanthanides or Rare Earth Metals | Phosphates, Inorganic | Substituted Triazines |

| Aliphatic Amines | Benzotriazoles | Epoxides | Neutral Organics | Phosphinate Esters | Triarylmethane Pigments/Dyes with Non-solubilizing Groups |
|---|---|---|---|---|---|
| Alkoxysilanes | Benzotriazole-hindered phenols | Esters | Nickel Compounds | Polyanionic Polymers (& Monomers) | Vinyl Esters |
| Aluminum Compounds | Boron Compounds | Ethylene Glycol Ethers | Nonionic Surfactants | Polycationic Polymers | Vinyl Sulfones |
| Aminobenzothiazole Azo Dyes | Cationic Dyes | Hydrazines and Related Compounds | Organotins | Polynitroaromatics | Soluble complexes of Zinc |
| | | | | | Zirconium Compounds |

**Category: <u>Acid Chlorides</u>** Environmental Toxicity

**Definition**. This category includes carbonyl chlorides (R-C[=O]Cl) and sulfochlorides (R-S[=O]Cl) where R may be either aliphatic or aromatic. Toxicity is limited by the fact that this class of compounds hydrolyzes and also, probably, if the octanol/water partition coefficient (Kow) is above a log Kow value of 8. It has been assumed that these compounds need to be absorbed to be toxic, therefore, compounds with MWs > 1000 will probably be excluded in the future once this assumption is confirmed with toxicity information. However, toxicity information is needed to confirm this assumption.

**Hazard Concerns**. Acute toxicity for three members of this category are available and all have been shown to be moderately toxic to aquatic organisms (i.e., acute toxicity values between 1 and 100 mg/L): benzoyl chloride, fish 96-h LC50 = 35.0 mg/L, an aromatic dicarboxyl dichloride, fish 96-h LC50 = 6.2 mg/L, and benzene sulfochloride, fish 48-h LC50 = 3.0 mg/L. All of these tests have been done with the static method using nominal concentrations. It is unclear just how acid chlorides are toxic to aquatic organisms. It is known that acid chlorides hydrolyze to the carboxylic/sulfonic acid and HCl. It is not known if the toxic effect is the result of (1) absorption of the acid chloride and hydrolysis within the membrane, or (2) the HCl produced from the hydrolysis. It is known that the carboxylic/sulfonic-acid hydrolysis products are of low toxicity.

**Boundaries**. There are no known lower boundaries. The upper boundaries will be based on $K_{ow}$ and MW when enough information is obtained. In general, when the log $K_{ow}$ value is < 8, the environmental base set of tests will be requested for aquatic releases and the terrestrial base set of tests will be recommended for terrestrial exposures. When the log $K_{ow}$ is > 8, testing will be requested until enough information is obtained to determine whether these compounds will have no toxic effects at saturation. Generally, members of this category will have MWs of less than 1000 but testing of members with a MW > 1000 may be requested to confirm whether acid chlorides have to be absorbed to be toxic.

**General Testing Strategy.** The testing strategy for acid chlorides will consist of two steps. (1) Hydrolysis as a function of pH at 25 C (40 CFR 796.3500) will be recommended. Depending on the outcome of this environmental fate testing and reassessment, (2) the aquatic base set of environmental toxicity tests will be recommended for aquatic exposures with the fish acute toxicity test done once or twice.

Chronic toxicity testing for aquatic organisms include: the fish early life state toxicity test, the daphnid partial life cycle toxicity test and the algal toxicity test.

The terrestrial base set of environmental toxicity tests (i.e., the early seeding growth test, the earthworm acute toxicity test and the soil microbial community bioassay) will be recommended for terrestrial exposures. Chronic toxicity testing for terrestrial organisms include: the plant whole life cycle test, the plant uptake test, and the soil microbial community bioassay.

**Category: <u>Alkoxysilanes</u>** Human Health, Environmental Toxicity

Definition. Any molecular structure containing one or more of the following reactive groups is considered to be a member of the category.

```
     | |
    -C-O-Si-
     | |
```
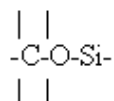
The "typical" new chemical of concern is a polymer with a substantial fraction of species with molecular weights <1000 and pendant trimethoxy- or triethoxysilane groups.

**Hazard Concerns.**

<u>Health</u> - Concern for lung toxicity from inhalation of vapors or aerosols is based on data for a number of low-molecular-weight alkoxysilanes. Trimethoxysilane (TMS) is clearly the most toxic member of the class causing irreversible lung effects at low doses, but the Agency does not consider it appropriate to use TMS as a regulatory benchmark for all alkoxysilanes. For trimethoxysilane monomers and polymers with a low trimethoxysilyl equivalent weight, a NOAEL of 10 ppm (about 11 mg/kg/day) based on a 90-day study with vinyltrimethoxysilane in monkeys is deemed an appropriate generic benchmark. Alkoxysilanes in which the alkyl substituent is **not** a methyl group do not appear to be as toxic as methoxysilanes. The New Chemicals Program currently uses a generic benchmark NOAEL of 75 mg/kg/day, based on a 90-day inhalation study with tri(isopropenoxy)silane, for alkoxysilanes other than methoxysilanes.

<u>Ecotoxicity</u> - Alkoxysilanes are highly toxic to algae and moderately toxic to aquatic invertebrates. For example, the daphnid 48-hr $LC_{50}$ for dimethyldiethoxysilane is 1.25 mg/L, and the 15-day algal $EC_{95's}$ for vinyltriethoxysilane, tetraethoxy-silane, and trifluoropropenyl(methyl)diethoxysilane are all approximately 10 μg/L.

**Boundaries.** Methoxy- and ethoxysilanes are presumed not to pose a hazard under any conditions if the equivalent weight is 5,000 and no more than 25% of species have molecular weights less than 1,000 and no more than 10% of species have molecular weights less than 500. For alkoxysilanes with alkyl substituents larger than propyl groups, the equivalent weight cutoff is 1,000. The degree of concern depends on the relative abundance of lower molecular weight species, but there is no molecular weight threshold above which there would be no concern.

To better define the boundaries of the category, EPA seeks testing on a limited number of alkoxysilanes that focuses on (1) the relationship between molecular weight (or alkoxysilyl equivalent

weight) and inhalation toxicity and (2) the importance of increasing alkoxy chain length in limiting toxicity.

## General Testing Strategy

The Agency recommends the following testing as appropriate to address health and environmental toxicity concerns for this category:

1. 90-day subchronic test in rodents by the inhalation route (40 CFR 798.2650).

2. Hydrolysis testing (40 CFR 796.3500). If $t_{1/2}$ is less than one hour, base set ecotoxicity testing (see "3," below) is conducted with the hydrolysis products only. If $t_{1/2}$ is greater than one hour, base set ecotoxicity testing is conducted with the parent material; the PMN submitter has the option of also testing with the hydrolysis products.

3. Base-set ecotoxicity testing to include fish (40 CFR 797.1400) using the static method, daphnids (40 CFR 797.1300) using the static method and algae (40 CFR 797.1050) using the static method, all nominal concentrations. Direct dilution of the test alkoxysilane and organisms is added within 10 minutes. The static-renewal method is used for fish and daphnid test, plus an additional fish test using aged stock solution.

Results of the acute ecotoxicity testing may trigger chronic fish (40 CFR 797.1600) and daphnid (40 CFR 797.1350) testing.

4. Physical-chemical or environmental fate testing including, as appropriate, melting point (40 CFR 796.1300) or boiling point (40 CFR 796.1220), water solubility (40 CFR 796.1840 or 796.1860), log $K_{ow}$ (40 CFR 796.1550, 796.1570 or 796.1720), vapor pressure (40 CFR 796.1950), direct photolysis and indirect photolysis (40 CFR 796.3765). Need for water solubility, log $K_{ow}$, and photolysis testing determined by outcome of above hydrolysis testing.

## Analog Identification Methodology (AIM)

The US EPA have no tools to identify or predict the toxicity of non cancer health endpoints. Instead read across arguments are used to assess and identify particular hazards. To facilitate read across a methodology know as AIM has been developed to help identify potential analogs of interest. The AIM approach comprises a large database of 31,031 compounds with publicly available toxicity data from a variety of sources. These compounds have been coded for the presence of 645 structural fragments and correction factors taken from the EPISuite KOWWIN program. Chemicals have also been coded with a ring index to enable faster retrieval.

During a search for potential analogs with available toxicity information, AIM assigns structural fragments, correction factors and the ring index to the compound of interest using the exact same algorithms as have been used to develop the database. The program uses a "three-pass" methodology to locate at least seven analogs and a maximum of 48 analogs.

- Pass 1- Analogs are selected when an exact match for all fragments, corrections and ring types occurs. If seven or more analogs are located, the search is terminated and the list of analogs is provided.
- Pass 2 - looks for additional analogs if less than seven analogs were located in pass 1. In this pass, analogs are selected based on two techniques. The first allows for different substitution patterns for alkyl substituents to be considered analogs. The second requires an exact match for only 262 structural fragments.
- Pass 3 - looks for additional analogs if less than seven analogs were located in pass 2. This pass allows halogen (chlorine, bromine, or iodine) substitutions between the compound of interest and analogs.

Known Limitations

- Rings - The current AIM methodology requires exact matching with respect to rings in the candidate compound. No substitutions are allowed (e.g. phenyl ring for a pyridine ring). The same number of rings is also required (e.g. dichlorodiphenylsilane will not be identified as an analog for trichlorodiphenylsilane). Methodology to remove this limitation is under investigation.
- Number of analogs included in the analog list - If Pass 1 locates seven or more analogs, Pass 2 and Pass 3 are not currently implemented; therefore, some additional good analogs may not appear in the results.

The current implementation of Pass 2 (and Pass 3) can add a sizeable number of analogs to the list. AIM is still in a beta test phase and extension and further development depends on input and feedback from beta testers. The tool itself is a simple means of identifying analogs (that have some toxicity data available) for read across. The tool does not categorise or rank the analogs returned. It is up to the individual user to decide when a specific analog is appropriate; no guidance to assist in this evaluation is available. The toxicity test data available is accessed in the form of hyperlink pointers. It is not structured in any way and cannot be downloaded into Excel or other tools for analyses and hypothesis testing. Some hyperlinks merely point to a general webpage e.g. IUCLID homepage or RTECS homepage so the user may need the appropriate licenses in order to be able to extract any available information. The pointer merely informs that there is a record for the chemical returned but not the type of data or its potential usefulness. AIM may be flexibly searched on the basis of structure, SMILES and CAS number though it cannot be searched by name.
There is no information to represent the chemical distribution within the AIM database. A visual representation to demonstrate the content of the database in terms of organics/inorganics or different classes of chemicals would be of considerable value to understand the scope/domain of the database.

References
http://www.epa.gov/oppt/newchems/pubs/chemcat.htm


**USE BY CANADA**

Environment Canada uses the following general rules of thumb but recognises that there will always be exceptions.

An analogue should preferably contain most, if not all, of the same structural features as the DSL (Domestic Sustances List) substance of interest.
- An analogue should have approximately the same molecular weight as the substance.

- An analogue should have water solubility similar to that of the substance of interest.

- For persistence, an analogue should have the same reactivity or stability as the DSL substance of interest.

- For an endpoint of interest, the relevant molecular descriptors of an analogue should be of comparable value to those of the substance.

It is recognised that different analogues may be selected for different endpoints, e.g. an analogue selected for a P endpoint may not be suitable for determining a B endpoint). Environment Canada and Health Canada rely on many of the on-line databases, but have also created extensive in-house databases for physical-chemical properties and toxicity that are searchable by structure using the Chemfinder software (http://chemfinder.cambridgesoft.com) or ISISBase (http://www.mdli.com/products/framework/isis_base/index.jsp). Most property and toxicity data for

new substances are stored in these databases and a large analogue database has been created by Environment Canada for DSL Categorisation.

**Use of read-across and categories by Health Canada**

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) requires categorization of the approximately 23 000 substances on the Domestic Substances List (DSL) prior to a legally mandated deadline of September 14, 2006.
The objective of categorization is to identify substances (on the basis of either exposure or hazard) that need further assessment. The two phases of assessment are screening assessment and in-depth assessment.
In order to efficiently identify and prioritize substances on the DSL that represent highest priorities from a human health perspective, a framework based on an iterative application of increasingly discriminating (i.e., simple and complex) tools for consideration of exposure and hazard was developed. The "simple tools" are sufficiently robust to address all substances on the DSL based on limited information; the "complex tools (ComHaz)"are more discriminating. Stepwise application of these tools minimizes over emphasis on data-rich compounds, while making optimum and efficient use of available information.
The complex hazard tool so-called ComHaz involves a hierarchical consideration of various sources of information (including data, [quantitative] structure–activity analysis and comparison with analogues) for a range of endpoints of toxicity.

**Complex Hazard tool (COMHAZ)**

In the Complex Hazard Tool (ComHaz), information on a variety of types of health effects identified from various sources is considered in a hierarchical manner.

This tool covers a range of toxicological endpoints considered in a stepwise manner and includes criteria specific to each endpoint. These endpoints have been selected based on consideration of potential public health impacts, as well as the likelihood of availability of relevant information. The endpoints, which are listed below, are considered in descending order

Endpoints included in the hierarchical approach are:
1. carcinogenicity;
2. genotoxicity;
3. regulatory/reference values;
4. developmental toxicity;
5. reproductive toxicity;
6. longer-term toxicity;
7. short-term toxicity; and
8. acute toxicity.

The available information on these effects is considered in sequential order, beginning with carcinogenicity. If any of the information satisfies the criteria for an endpoint, the substance is prioritized for further consideration in subsequent stages, which include a preliminary assessment of weight of evidence for qualitative endpoints and development of measures of exposure–response for critical effects. If the criteria are not satisfied or insufficient data relevant to that endpoint are identified, the available information on the next endpoint is considered.

For regulatory/reference values (generally based on longer-term studies), longer-term toxicity, short term toxicity and acute toxicity, if information is sufficient but does not meet the criteria, it is not necessary to consider steps lower in the hierarchical approach, and the substance can be "Set Aside"

for no further consideration at this time. Substances can be "Set Aside" based on regulatory or reference values, because these values are generally based on lowest- or no-effect levels for critical effects identified through comprehensive assessments of the available data.

Setting substances aside on the basis of longer-term, short-term and acute toxicity is predicated on the toxicological principle that the amount of a substance required to induce health effects generally decreases with increasing duration of exposure, and more sensitive effects are likely to be discernible in longer-term studies. Thus, if a substance is deemed not to be of concern for longer-term toxicity (on the basis of comparison of adequate information with the quantitative criteria in ComHaz), it is unlikely to be of concern for effects induced following exposures of shorter duration.

Once a substance is prioritized for further consideration on the basis of a given endpoint, there is no need to consider available information on endpoints that are lower in the sequence at this initial stage. This approach permits the initial prioritization of a large number of substances in an efficient and effective manner. While a substance may be prioritized for further consideration without evaluation of the data available for every endpoint in the hierarchy, data on all relevant endpoints will be considered during subsequent phases. If the available information on a substance does not meet the criteria specific to any of the components considered in the hierarchy, the substance is considered to be of low toxicity based on this conservative tool, and it is "Set Aside" at this time, with no requirement for further consideration. However, in some cases, substances "Set Aside" at this time may be reconsidered at some later date in the light of additional data.

**Hierarchical Consideration of Sources of Information**

Various sources of toxicological information are considered to determine if a substance meets the endpoint-specific criteria proposed for ComHaz. These sources of information are also considered in a hierarchical fashion in descending order of degree of confidence, in that acceptable assessments of international or national agencies and secondary reviews are first consulted, followed by original study accounts, predictions of quantitative structure–activity relationship (QSAR) models, information on chemical substructures of concern and analogues or surrogates (Figure 1)

If no relevant toxicological data are identified, QSAR models are used to predict the likelihood that a substance will induce adverse effects on health. Of the various commercially available QSAR models identified, those proposed for use in the first stage of ComHaz currently include the statistically based TOPKAT and/or CASETOX models for carcinogenicity, genotoxicity, developmental toxicity, chronic toxicity and acute toxicity.

**Use of SARs and read-across**

In cases where insufficient information from assessments or reviews of other agencies, primary study accounts or QSAR predictions is available to permit a conclusion with respect to initial prioritization of a substance on the basis of the toxicological endpoints included in the proposed hierarchical scheme (including reference values established by other agencies), substances are examined to determine if they contain chemical structures or structural subfragments that have been correlated with toxicity, based on comparison with other sources of information. These sources include non-quantitative structure–activity relationship (SAR) models (e.g., automated expert systems such as DEREK), lists of chemical substructures of concern compiled by other agencies (excluding those identified by DEREK) (see Table) and extrapolation of toxicity information on analogue or surrogate substances identified using relevant databases and automated structure or substructure search engines (e.g., Accord, Leadscope). Substances containing substructures of concern associated with endpoints considered relevant in the hierarchical approach described above or for which appropriate analogues or surrogates are associated with these effects are prioritized for further consideration. Although these

sources of information are consulted only if the results of QSAR predictions are insufficient, this does not imply that there is greater confidence in predictions from QSAR models versus chemical structures of concern, automated expert systems or extrapolations from analogues. Many of the principles intrinsic to these sources of information are also incorporated into the commercial QSAR models. However, the ease of running and validating predictions from the commercial QSAR models and the range of endpoints (some for which predictions are quantitative) covered by these systems facilitate their direct incorporation into the ComHaz endpoint hierarchy so that large numbers of substances can be more efficiently evaluated.

### Considerations Relevant to Specific Groups of Substances

### Organic and Inorganic Acids, Bases and Salts

The approaches to the application of ComHaz to organic or inorganic acids, bases and salts are dependent upon whether the substance in question is considered to be soluble or not. For the purposes of applying ComHaz, an organic or inorganic acid, base or salt is considered to be soluble if its measured or predicted solubility is $\geq 1$ mg/litre. Alternatively, a qualitative determination that an acid, base or salt is soluble or very soluble may be made based on other information such as empirical data, thermodynamic calculations and computer modelling with the application of scientific professional judgement.

When applying ComHaz, it is assumed that soluble acids, bases and salts can exist not only as intact substances, but also in alternate forms. For example, a soluble salt could be intact, 100% ionized or exist as the corresponding acid or base. When possible and considered appropriate, the alternate forms of soluble acids or bases and their salts can be grouped in order to take advantage of the data available on all of the substances in the group before making a determination of whether any substance in the group meets the criterion for a specific endpoint in ComHaz. For example, when insufficient information is available to reach a decision for a specific endpoint in the ComHaz hierarchy for a soluble acid, base or salt then data and QSAR model predictions for the alternate forms of the substance may be considered. In addition, when extrapolating from data on an alternate form of an acid, base or salt, previous decisions to either prioritize the alternate form for further consideration or set it aside for no further action may be taken into consideration

Acids, bases and salts that are not soluble are considered in ComHaz in the same manner as simple organic substances. However, if inadequate data or model predictions necessitates the application of surrogate or analogue approaches, then preference is given to extrapolations based on data from surrogate or analogue substances that are not soluble.

Scientific professional judgement must be considered when determining whether it is appropriate to reach a decision on an acid, base or salt for a specific endpoint in ComHaz based on an extrapolation from an alternate form of the substance, or surrogate or analogue substances.

### Mixtures

If ComHaz is applied to a mixture and relevant data on the mixture as a whole are not identified for a given endpoint in the hierarchy, the individual components of the mixture may be considered separately in a manner similar to that outlined above for the alternate forms of a soluble acid, base or salt. Also, similar to the alternate forms of a soluble acid, base or salt, previous decisions to either prioritize a mixture component for further consideration or set it aside for no further action may be taken into onsideration when applying ComHaz to the whole mixture.

As outlined above, the application of ComHaz involves the comparison of information on a series of toxicological endpoints relevant to human health with endpoint-specific criteria that can be qualitative (e.g., carcinogenicity/genotoxicity) or quantitative (e.g., repeated-dose toxicity). For endpoints with qualitative criteria, where possible, in subsequent initial phases of screening, the data will be considered in a preliminary weight of evidence approach, the objective of which is to additionally discriminate priorities for further consideration without imposing undue workload, the latter being more appropriate to subsequent phases of screening and in-depth assessment.

**References**

Health Canada (2005). A Proposed Integrated Framework for The Health-Related Components Of Categorization Of The Domestic Substances List Under CEPA 1999. Health Canada, Ottawa. June 2005.

Figure 1    ComHaz hierarchical consideration of sources of information
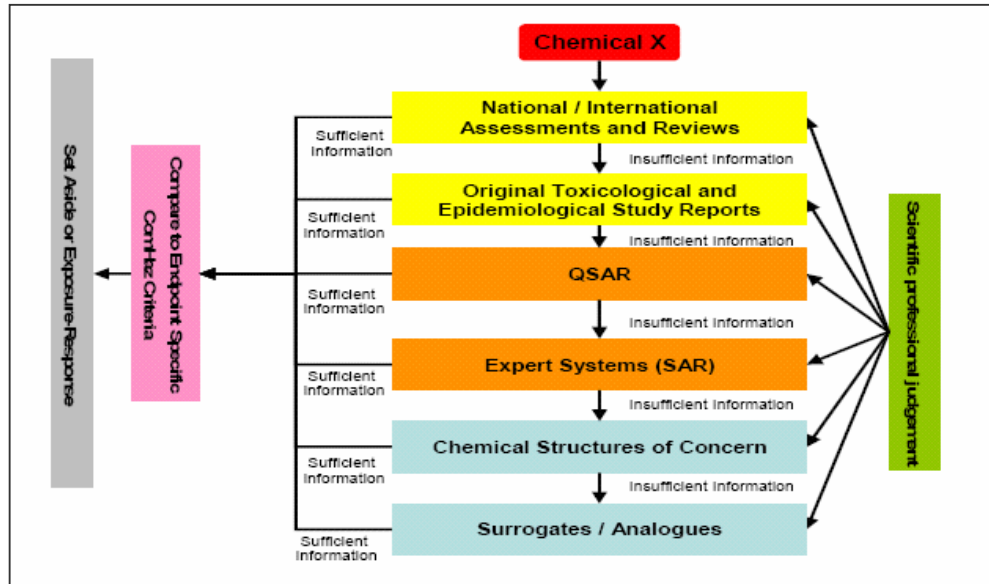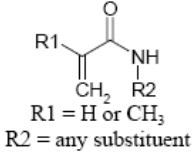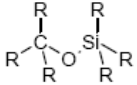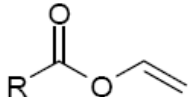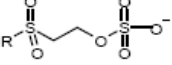
Figure 2　　　　SARs used in ComHaz

### Table AII-1. Chemical Substructures of Concern

| Category[a] | Structure | Description | Potential Endpoint Associated with Structure[b] |
|---|---|---|---|
| Acrylamides | R1, O, NH, CH₂, R2, R1 = H or CH₃, R2 = any substituent | Any chemical with the structure indicated | Carcinogenicity, mutagenicity, reproductive toxicity, developmental toxicity, neurotoxicity |
| Alkoxysilanes | R, R, R–C–O–Si–R, R, R | Any structure containing one or more of the indicated reactive group | Lung toxicity |

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| Anhydrides, Carboxylic acid |  | Any structure containing one or more carboxylic acid anhydride groups | Developmental toxicity, reproductive toxicity, pulmonary sensitization |
|---|---|---|---|
| Ethylene glycol ethers | <br><br>n=1, 2, or 3<br>R1= alkyl C7 or less or phenyl or alkyl substituted phenyl<br>R2= H or alkyl C7 or less | Indicated structure | Reproductive toxicity, developmental toxicity, systemic toxicity (blood, kidney, liver), immunotoxicity, central nervous system depression |
| Hydrazines and Related Compounds |  | Any structure containing one or more of the indicated groups | Carcinogenicity, systemic toxicity (blood, kidney, liver), central nervous system depression |
| Hindered Amines |  | | Immunotoxicity, systemic toxicity (blood, liver, gastrointestinal tract), reproductive toxicity |
| Phenolphthalein |  | Any chemical containing the phenolphthalein structure | Carcinogenicity |
| Triarylmethane pigments | <br>R= H, CH₃, C₂H₅ | Derivatives of triphenylmethane or diphenylnaphthylmethane<br><br>Amine groups (primary, secondary or tertiary) or hydroxyl groups must be present on the aromatic ring positions *para* to the methane carbon | Carcinogenicity, developmental toxicity, reproductive toxicity |

| Vinyl Esters |  | A carboxylic acid ester with at least one vinyl group (CH₂=CH-) attached to an organic acid group (RCOO-) | Carcinogenicity, mutagenicity, reproductive toxicity, neurotoxicity |
|---|---|---|---|
| Vinyl Sulfones | vinyl sulfone  sulfatoethyl-group  | Any structure with a vinyl sulfone group or sulfatoethyl-sulfonyl group (typical vinyl sulfone precursors) | Carcinogenicity, mutagenicity |

**RECOMMENDATIONS FOR FURTHER DEVELOPMENT OF GUIDANCE BY EU QSAR WORKING GROUP**

This review aims to outline and summarise some of the practical experiences from the major Regulatory agencies as well as the guidance so far developed by the OECD.

This has helped inform where the strengths and limitations exist and where there are opportunities that could be further explored.

Working in partnership with stakeholders from Health Canada and the US EPA would help to gain valuable practical experience about how category proposals are typically undertaken.

Recommendations for next steps should include:

- Review of the available approaches and/or tools for identifying analogs including descriptors for chemical similarity/structural keys/pharmacophores/fingerprints
- Investigate the feasibility of how different chemical similarity descriptions can be applied to the identification of chemical groupings using one or more existing regulatory inventories
- Based on such exploratory research work, solicit feedback from the working group to establish a "toolbox" of available and recommended approaches
- Establishment of a small drafting team (comprising members from the ECB, the QSAR working group as well as stakeholders from Canada, US, OECD) to write a guidance document. This document should also include a compendium of case studies highlighting the strengths and limitations of a given approach for a specific purpose/endpoint