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# Non-testing strategies – a tiered approach

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## Purpose

Computational methods are sometimes considered a black box able to deliver automatically endpoint values plus reports including sufficient methodological information (QMRF, QPRF) just on the basis of a chemical structure information. However this may be eventually achieved a tiered approach is more realistic as experienced on the basis of actual REACH registration work done in a consultancy. Prior to any computation waiving, mode of action MoA, and speciation should be checked.

## 1. Not assessing - Waiving of an endpoint information requirement?

Test protocols are designed for soluble, non-volatile organic small molecules, but may not be able to produce meaningful data for
- Inorganic chemicals or silicium-organic compounds
- Macromolecules (e.g. Peptides)
- Nanomaterials
- Pre-drugs, unstable compounds

Waiving is frequently required for but is not restricted to industrial chemicals and can be justified:
- Legally based (e.g. “column 2” REACH Annexes VII to X)
- Scientifically based (obtainable results useless for assessment)
- Technically based (valid study not feasible)
- Exposure based, e.g. when the Exposure model indicates PEC below an established Threshold of Toxicological Concern (TTC)

## 2. Not testing - Waiving of a study requirement?

Legal frameworks generally consider well defined endpoint information requirements and standard test protocols to produce the data. In an integrated testing strategy (ITS) the synopsis of the available information may enable the assessment of one endpoint by data from a different one.

Such **Endpoint-Analogy Read-Across** may be based on
- Exposure time (chronic tests can cover acute effects)
- Exposure route, e.g. equilibrium partitioning method (EPM) or route-to-route extrapolation (Oral ↔ Inhalation, Oral ↔ Dermal)
- Parallel observations, e.g. is a long-term fish toxicity not required in case an existing bioconcentration study evidences no effects at a level above the algae NOEC or a hydrolysis study is obsolete if sediment simulation data show persistence

## 3. Analogy approach

**(1) Principles**

Analogy regards the chemical species whereto an organism is exposed and the relevant bioavailability. This means e.g. the availability at the target structure (receptor) site when such receptor contact is the starting point of the adverse outcome pathway (AOP) of the toxicological response

Thus such analogy approaches intend not to use using target chemical test data but experimental evidence from

a **test surrogate** (identical chemical species, analogue bioavailability), e.g. in a pre-drug case test information from a readily formed metabolite, or in an actual exposure case results from a test item with a different non-toxic counterion, or

an **analogue material** (analogue chemical species, identical bioavailability) in a point-to-point read-across from only one chemical up to a trend analysis (QSAR) within a larger group containing as well less homogeneous compounds.

When evaluating chemically similar molecules it may turn out that
- data for only one substance exist or can be used (access, intellectual property)
- the analogues do not fit into one category (group of read across substances with common MoA and a single determinant) or
- a category can be justified, which means the analogue materials fit into a clear trend analysis relation or function (see below, section on QSAR).

Assessing on the basis of activity information from only one analogue molecule is generally weak and additional evidence may be given, e.g. by showing that computation and measurement of the analogue material data are in satisfying agreement.

Expert statement discussing all parts of the molecule in question is mandatory.

Read-across can be
- quantitative (considering isomolarity) or
- non-quantitative (e.g. only evidencing absence of a particular MoA), which may justify assignment of baseline toxicity considering chronic body burden (CBB)

Due to the uncertainty of every read across approach, concluding on a weaker isomolar effect of the target chemical is critical as this would mean **extrapolation**. A solid rationale is required e.g. likelihood of lower bioavailability due to fugacity or biological inactivation (transformation, metabolisation, trapping).

The assignment of a mean value is more easily justifiable as it means **interpolation** or **bridging** (i.e. at least two values bracket the assigned one).

**(2) Read-Across Types**

Using **test surrogate** data (actual exposure to the relevant target chemical species):
- Salt to acid and vice versa
- One salt to salt with a different anion or cation
- Racemate data to stereochemically defined enantiomer target chemical
- Pre-drug case, i.e. quick generation of target chemical

Alternatively **analogue materials** having only insignificant changes in their chemical structure and/or identical mode of action (AOP, 3 “-omics”) can be used.

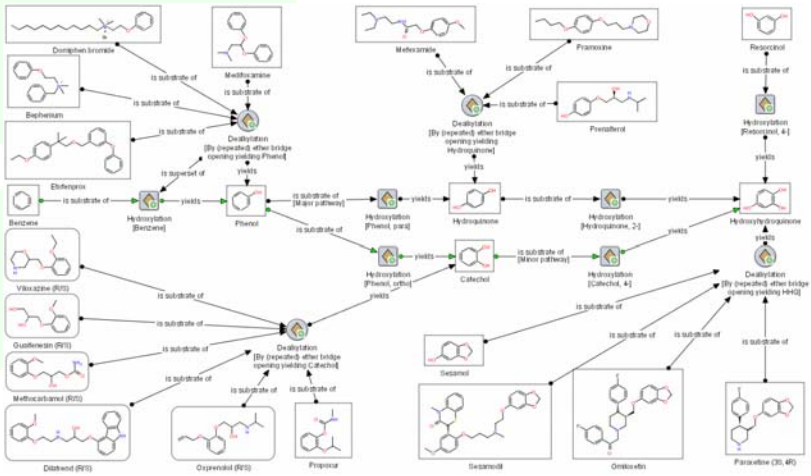
**(3) The Category**

However similarities based on structural factors are important, the statistical similarities (e.g. Tanimoto) covering the whole molecule may be insufficient in case only cleaving of a minor part from larger molecules sets the toxicologically relevant molecule free. Once a hypothesis exist, true substructure search (SMARTS) may be more relevant.

A chemical category is (to be) defined with regard to a
I. Category rationale (common structural elements)
II. Particular endpoint or a group of endpoints whereto an AOP leads
III. Scope (applicability domains for category and prediction)

A number of categorisation criteria as e.g. the functional group based ECOSAR categories can be applied by software (OECD Toolbox) but need to be verified / discussed and combined with other mechanistically relevant properties (e.g. protein binding)

**Figure:** Pathways of molecules potentially cleaving Hydroxyhydroquinone (HHQ) or a precursor of it are suggested forming a category with regard to benzene-typical chronic endpoints, which are assumed to be caused by HHQ. A computer based intelligence platform (OKAPI) with an implemented ontology for the knowledge management of ADR mechanisms (Hug et al 2003) was used for hypothesis formulation. The benzene human metabolism is known (Snyder & Hedli 1996). The knowledge management connects typical endpoints, i.e. not furthermore differentiated neural tube defects and major cardiac defects including but not restricted to ventricular septal defects in the newborns, with chronic low dose benzene exposure via public drinking water contamination (Bove et al 1995). Lupo et al (2010) confirmed statistical correlation of benzene exposure to spina bifida, while anencephaly did not correlate. FDA (2005) considered the coincidence between one of these endpoints (cardiac defects, most of them were atrial or ventricular septal defects) and the intake of paroxetine in gravidity to be significant. The paroxetine metabolic pathway taken from the US drug approval documents is considered to converge into the one of benzene. Similar crossing pathways have been found with different evidence levels for a number of drug active ingredients and other substances (Wess et al 2005).



**(4) Computation – QSAR/QSPR**

Requirement for Quantitative SAR is the proportionality to a determinant, which is mostly a fugacity constant (Kow, Kaw, Koa, Kmw), while molecular weight and bulkiness parameters are more important as domain cut-offs.

In case trend analysis in a category is possible with sufficient statistical accuracy, the target chemical activity can be computed according to the function.

Whether a number of chemical structures and endpoint data can be used altogether in one training set or need to be separated in categories first (top-down approach) is dependent of the MoA leading to the endpoint. While properties may be predictable by summarizing over structural fractions (QSPR), biological endpoints require a comparable MoA. Nonetheless molecular descriptors selected by statistical means can be used for (eco)toxicological endpoints (T.E.S.T, Lazar, DEMETRA). Such “statistical” QSAR not generally met the 5th OECD principle demanding a mechanistic interpretation, but may deliver category criteria.

## 4. Mixture effect calculation

The relevance of known impurities should be assessed before finally concluding. In the case of a mixture, multi-constituent or UVCB substance the effect level can be estimated using data and QSAR from the constituents. The Independent Action (IA) model calculates combined effects of components with different MoA, while the concentration addition (CA) model is more conservative assuming a contribution of the compounds to the same MoA. It may be considered to calculate the combined effect of substances with a common MoA and than integrate over such group effects using IA. The required information for the hazard assessment are same type threshold value for all components (IA) or the dose response curves (CA). As this function is often unavailable the risk can be assessed by adding the hazard indexes (HI) when CA is assumed. The maximum cumulative ratio (MCR), i.e. sum HI / max HI) indicates the importance of a mixture component. Biotic ligand models (BLM) apply to metal mixtures and are based on interaction data.

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