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Non-testing strategies

Grundprinzipien und Ansätze

R. Arno Wess
Regulatory Ecotoxicologist
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Non-testing alternatives / overview

1. Not assessing on an endpoint (waiving) of the submission item
   - e.g. biodegradation or persistence of inorganic substances
2. Not testing on an endpoint and assessing on the basis of evidence from available tests with the submission item for a different endpoint
   - Route-to-route extrapolation (ITS, EPM), or (more) chronic instead of (more) acute data; e.g. 90 day Toxicity covering 28 day toxicity; sediment simulation study covering absence of hydrolysis
3. Not using target chemical test data but from
   - Test surrogate (identical species, analogue bioavailability)
     - Pre-drug case (metabolite); Actual exposure case
   - An analogue material (analogue species, identical bioavailability)
     - Point-to-point (read across); Trend analysis (QSAR, QSPR)
4. Not using mixture / multi-constituent / UVCB test data but from its constituents
   - Combined action (CA) or Independent action (IA) model

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Waiving

If measurement is not helpful for assessment

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Waiving possibilities

Test protocols are designed for more or less soluble organic small molecules, but may not be able to produce meaningful data for

- Inorganic chemicals
- Macromolecules (e.g. Peptides)
- Organisms
- Pre-drugs
- Unstable compounds

Waiving is frequently required for but not restricted to industrial chemicals and can be based on:

- Legally based (e.g.“column 2” REACH Annexes VII to X)
- Scientifically based (obtainable results useless for assessment)
- Technically based (valid study not feasible)

More than just one waiving reason?
Both, Waiving & assessment?

It may happen that one case the assessment of / testing for an endpoint can be based on more than only one principle:

- Legally based (correct juristic citation)
- Scientifically based (often the reason for a legally based waiving, but the argumentation may be given in addition to the citation of the law)
- Technically based (a test protocol is not designed for and can not be adapted for a particular substance – thus the precise reason should be given, e.g. a compound is very unstable – nonetheless in addition to the waiving the risk may be assessed on the resulting transformation products)

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Analogy approach – Read across

Analogue material or test surrogate

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Data from only one analogue or inhomogeneous analogues

When evaluating chemically similar molecules it may turn out that

- **Data for only one substance exist or or can be used**
- **The analogues do not fit into one category (group of read across substances) or**
- **A category can be justified, this 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.

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 thus be

- **Quantitative – consider isomolarity**
- **Non-quantitative (e.g. absence of a particular MoA) – assign at least baseline toxicity / CBB**

Interpolation, extrapolation

Due to the uncertainty of every read across approach

- **Conclusion on a weaker isomolar effect of the target chemical is very critical**
  - **extrapolation**
- **In case of available data from two analogue materials the assignment of a mean value may be justifiable as it could be regarded as**
  - **interpolation**
- **But a solid rationale, e.g. likelihood of biological inactivation (transformation, metabolisation) or reduced bioavailability is required.**

Types of Read across – Source and target chemical pairs

1. Using test surrogate data

- **Salt to acid and vice versa**
  (cave: Cut-off limits, as acids have less MW)
- **One salt to salt with a different anion or cation**
  (cave: Cut-off limits, in case data from less MW ion are used)
- **Racemate data to stereochemically defined enantiomer target**
- **(Cave: Enantiomers have the same fugacity but not necessarily the same bioavailability as transformation/metabolisation may be different; Consider all specific activity from the target chemical, except minor activity of its enantiomer(s) is proven; vice versa NOT POSSIBLE)**

2. Using analogue materials (having only insignificant changes in their chemical structure)and/or identical mode of action (AOP, 3 "-omics")

- **Consider isomolar effects**
- **Sound argumentation for irrelevance of structural differences required (e.g. using comparable that comparable changes at a different basic structure revealed in actual tests insignificantly different isomolar effects)**

Types of Read across – From one exposure to another

1. Ecotoxicology (further data required on fugacity, solubilities)
    - EPM
    - BLM
2. Toxicology (further data required e.g. on adsorption, skin penetration, particle size)
    - Oral ↔ Inhalation
    - Oral ↔ Dermal

Uncertainty in different analogue material read across

At least the (larger) CI95% uncertainty of the source chemical value(s) is to be assigned to the target chemical value case of point-to-point read across

The establishment of a trend line function allows to use the uncertainty according to the external validation

With decreasing uncertainty the analogue material read-across options are listed:

1. One analogue material (point-to-point)

2. Two analogue materials, no category or trend analysis function statistically not acceptable (most critical point-to-point)

3. Two or more analogue materials, trend line determinant value of target chemical exceeds data range (extrapolated trend line-to-point)

4. Two (bridging) or more analogue materials, trend line determinant value of target chemical within data range, i.e. interpolation (trend line-to-point)

5. Calculation according to category QSAR/QSPR (computation)

6. Calculation according to a valid category QSAR/QSPR in accordance with all OECD criteria (valid QSAR/QSPR determination)

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Category approach

Baseline or mode(s) of action

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Category – is structural analogy enough?

A chemical category is (to be) defined with regard to

- a particular endpoint or a
- a group of endpoints

Predefined categories exist (e.g OECD, ECOSAR, Cramer rules etc.) but need to be verified/discussed as particular properties may be relevant

- Important for chronic effects, e.g. ED

Requirement for QSAR is a (quantitative of course) relationship

- In case a trend analysis in a category is possible with sufficient statistical accuracy the target chemical activity can be computed according to the function of one (or more) predictor
  - Generally a fugacity descriptor is the determinant
- Else: Qualitative conclusion only (absence of specific activity)

Quantitative and qualitative determinants

Generally a fugacity descriptor, obtained by physical/chemical property measurement whereof the n-octanol solubility may be of particular interest, is the quantitative determinant for activity

- Kow ($c_{octanol}/c_{water}$)
- Koa ($c_{octanol}/c_{air}$)
- Kaw (c_{air}/c_{water} or H‘ the dimensionless Henry constant)

Domain cut-offs typically are defined by

- Bulkiness parameters (MML, Dmax, Dmin?, Dmax-avr?)
- Molecular weight

But Skin penetration kinetics … (diffusion depends on MW)

Inorganic categories

Inorganic chemicals can often not be degraded in the sense than chemical bounds were break not to be restored in biota or in the environment

- Speciation equilibration
- Geochemical conditions as determinants for bioavailable species

As long as no organometallic compounds were formed by

- Metabolisation or
- Transformation

The emission of or the exposure to one of the possible species may result in rapid presence of other category member species

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The computational approach – QSAR, QSPR

Quantitative prediction

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Application – Endpoint and Trainig set dependent

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 is dependent of the mode(s) of action leading to the endpoint

- Properties often can be calculated on the basis of fragment contribution
  - QSPR often with thousands of data points in training sets
- For biological activities a common MoA is required
  - Otherwise no trend analysis function with sufficient statistical accuracy can be found

OECD criteria mandatory for regulatory use

In November 2004, the 37th OECD's Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (Joint Meeting) agreed on the OECD Principles for the Validation, for Regulatory Purposes, of (Q)SAR Models

The agreed OECD principles are as follows:

"To facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information:

- **a defined endpoint;**
- **an unambiguous algorithm;**
- **a defined domain of applicability;**
- **appropriate measures of goodness-of-fit, robustness and predictivity;**
- **a mechanistic interpretation, if possible."**

OECD (2006), Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals, Series on Testing and Assessment, No. 58, OECD, Paris, 79pp., http://www.oecd.org/document/30/0,2340,en_2649_34365_1916638_1_1_1_1,00.html, accessed 6 February 2007.

OECD criterion 1

The End-point measured must be transparently defined

And of course the model algorithm must be developed for the same endpoint as the one intended for substitution of measurement with the target chemical.

Inconvenience: The model can be constructed using data measured under different conditions and various experimental protocols.

- **E.g. U.S. EPA ECOSAR calculates algal effects according to the OPPTS standards, i.e. 96 h exposure**
  - **Using such prediction without further ado as substitute for a 72 h exposure measurement is a clear violation of this OECD principle!**

Tichý M & Rucki M (2009) Validation of QSAR models for legislative purposes - Interdisc Toxicol 2(3):184–6, doi: 10.2478/v10102-009-0014-2

OECD Environment Health and Safety Publications, Paris, France, (2007) GUIDANCE DOCUMENT ON THE VALIDATION OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIP [(Q)SAR] MODELS- Series on Testing and Assessment No. 69

OECD criterion 2

The algorithm used for construction of a model must be univocally given.

Cave: “Statistical QSAR”

Inconvenience: It is known that this information is not given with many commercial models. Information is not given, the organizations selling the model do not provide the information and it is not open to public. There are commercial reasons.

- **Derek, TOPCAT etc.**
  - **However the manufacturers provide some method description in “reporting formats“**

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OECD criterion 3

The applicability region must be defined.

Each QSAR model is directly joint with chemical structure of a molecule, with physicochemical properties of the substance and mechanism of the effect, which were used for a construction the model. All these parameters had values from some quantity to other one, depending mostly on availability of data and possibilities of their measurement. The same fact is valid for the biological test object, conditions of experiments, etc..

- **Applicability domain**
  - **Descriptors: MW, fugacity properties, bulkiness, functional groups and their number and/or combinations etc.**
- **Predictivity domain:**
  - **Range of endpoint values in training & validation**

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OECD criterion 4

Suitable statistical evaluation of the models must exist.

Internal and external validation should be applied. The external validation with independent series of data should be used. If not possible cross-validation can also serve. The statistical indices joint with predictability and reproducibility of the model must be calculated.

- **The standard deviation of the predicted value is mandatory in order to compare with measured data and/or cut off values.**

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OECD Environment Health and Safety Publications, Paris, France, (2007) GUIDANCE DOCUMENT ON THE VALIDATION OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIP [(Q)SAR] MODELS- Series on Testing and Assessment No. 69

OECD criterion 5

A mechanism of the end-point effect should be given.

If known. This principle should push authors of the model to consider an interpretation of molecular descriptors used in construction of the model in mechanism of the effect and this study should be documented.

Cave: “Statistical QSAR”

- **Just statistical correlation can be reached by increasing the number of phys-chemical constants.**
- **Therefore the mathematical algorithm is mandatory as internal and external validation(s) are!**

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Reporting formats – do not replace criteria fulfilment

In order to assure and facilitate structured information reporting for the validity / OECD criteria compliance check:

The QSAR Model Reporting Format (QMRF)

– version 1.2

The QSAR Prediction Reporting Format (QPRF)

– version 1.1

ECHA (2008) Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals, R.6.1.9 & R.6.1.10

What if one or more criteria are not fulfilled?

In case not all criteria are clearly fulfilled

- **The computed value can not without further ado substitute an experimental result of the submission item for regulatory purposes**
- **The assessment of impurities, transformation products, and metabolites may use such figures if the result indicates a comfortable safety margin**

Provided that

- **further evidence is available and/or**
- **the application of an additional AF (in all external validations of high level toxicological endpoints the predictions of "statistical QSAR" was in the range of ±Value•AF, if AF≤100) would not produce a value of concern**

acceptance or reduced / confirmatory testing may be discussed with authorities (testing proposal under REACH)

An important check

Available measured data on related endpoints should be in agreement with the employed QSAR / QSPR approach!

If not predictions basing on the same training set and/or algorithm are questionable

- **E.g. of the Ames test Mutagenicity prediction is wrong, the Carcinogenicity prediction is uncertain**
- **If water solubility prediction is wrong, Kow estimate is in question**
- **If the melting point prediction is not acceptable it is probably not better for the boiling point**
- **If biodegradation is erroneous the BCF calculation is probably not reliable**

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Pitfalls

Estimation failures

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Pitfalls

1. No analogy covers

- **Different transformation**
- **Different metabolisation**
- **Different speciation**

2. No QSAR is applicable if

- **The target chemical is not stable, i.e. forms transformation products and/or metabolites**
- **The training dataset is based on guessing rather than a category**
- **Relevant species do not fit in the applicability domain**
  - **Particularly relevant for ionizable substances**

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Mixture effect calculation

Estimation of summtion effects

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Calculation of Mixture effects

Required information: Independent or Combined Action?

- **IA**
- **CA (dose response curve required)**
- **MCR – often one or e few chemicals trigger the effect**
  - **Review data show: Environmental mixtures had MCR<6, thus an additional AF=10 would cover the risk**

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A final remark

Fight for non-testing?

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Remember

Replacement, Reduction and Refinement

of

vertebrate testing

is not just a good idea

– its the law (e.g. REACH).

However the registration process may regulated by different directives (PPP, biocides etc.) REACH regulation (a European law) applies directly and in principle to all chemicals ...

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