European Food Safety Authority

Non-testing methods, metabolites and formulations

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Non-testing methods

Supplement additional information
NOT in lieu of information demanded by the data requirements
Similarity principle

Danger of under-estimation of toxicity
Burden of proof:
 acceptable for positive findings (to avoid testing)
 not acceptable for negative findings
  - not all MOA covered by non-testing methods

Weight of evidence approache:
combining (Q)SAR/Expert system output with read-across data, TKTD info etc

Guidance on QSARS

Variable quality of input data used to develop and validate the model (VALIDATION)

Model simplification (APPLICABILITY DOMAIN)

WITHIN applicability domain, an estimate of toxicity from the training set averages the uncertainty and is more reliable than the information from any one piece of data in the training set

ECHA 2008
'Guidance on information requirements and chemical safety assessment Chapter R.6: (Q)SARs and grouping of chemicals'

When can a QSAR prediction be used?

Step 1

Assess validity of the QSAR model

No absolute measure – relative process
Five principles (OECD 2007)

1) Defined measureable endpoint and experimental protocols
2) Unambiguous algorhythm
3) Defined chemical domain of applicability
4) Measures of goodness of fit, robustness and predictivity
5) Mechanistic interpretation

OECD (2007) Guidance document on the validation of (Quantitative) Structure-Activity Relationship ((Q)SAR) models. ENV/JM/MONO(2007)2.

Step 2

Reliability of the individual model prediction

1) Is the chemical of interest within the applicability domain of the chosen model?
2) Is that applicability domain relevant to the regulatory purpose?
3) How well does the model predict activity of chemicals that are similar to the chemical of interest?
4) Is the model estimate reasonable considering other information?

Step 3

Adequacy of the QSAR information for making a regulatory decision

Key principles (ECB 2005)

1) Proportionality:
   amount of information - severity of decision
2) Caution/conservatism:
   amount of information – consequences of getting it wrong
3) Proximity:
   confidence and precision – closeness to a regulatory cut-off

Common to all human decision making!

ECB (European Chemicals Bureau), 2005. Scoping study on the development of a technical guidance document on information requirements on intrinsic properties of substances. TAPIR report

QSARs for Aquatic Toxicity

ECOSAR (US EPA)

(Q)SAR Application Toolbox (OECD)

DEMETRA (EU)

The Danish (Q)SAR Database

TOPKAT

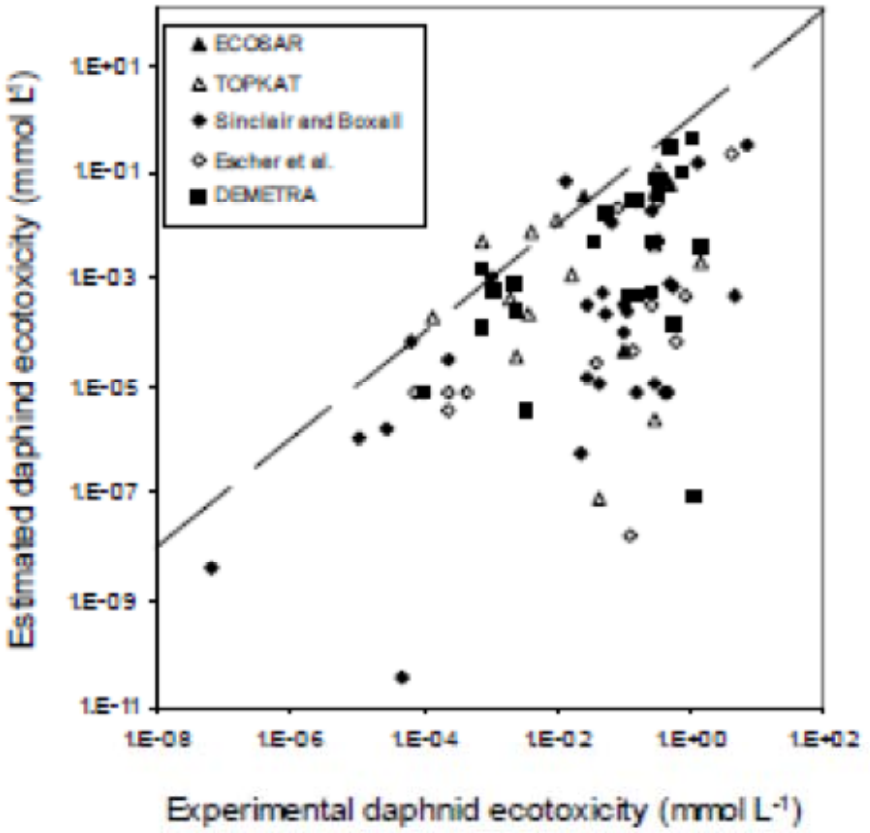
ChemProp

Model Inventory available at EC JRC Website

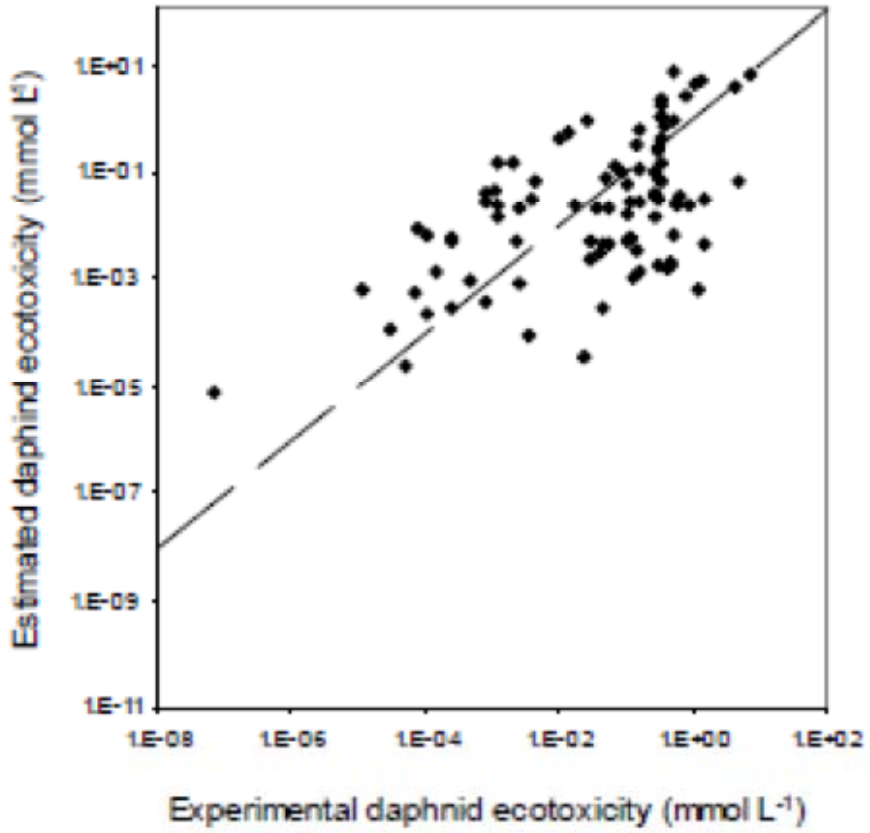
http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsar-inventory

Comparison of (Q)SAR model outputs

A. Most potent estimates



B. Geometric mean



Greater potential for underestimation of potency with aggregation methods

Recommendation to use most potent estimate pending further validation

READ-ACROSS for metabolites

Molecular structure (toxophore present/intact?)

Occurrence in tests with a.s. or major metabolites

Available information on related compounds

Relationship between toxicity of metabolites and parent compounds

Escher approach:
Generate TR from baseline toxicity and experimental toxicity of parent

$$\text{Toxic ratio (TR)} = \frac{LC/EC_{50,baseline}}{LC/EC_{50,experimental}}$$

Manipulate modelled baseline toxicity of metabolite with TR of the parent compound to generate estimate of the specific toxicity of the metabolite

$$\log\left[\frac{1}{LC/EC_{50,specific}}\right] = \log\left[\frac{1}{LC/EC_{50,baseline}}\right] + \log TR_{parent}$$

Decision scheme for use of non-testing systems

1. Is the (Q)SAR model valid (5 OECD principles, assessment values for predictiivity eg Q2, CCC, SD)?

   YES – Go to 2
   NO – (Q)SAR should not be used - consider other model

2. Is the chemical within the applicability domain of the model

   YES – Go to 3
   NO – (Q)SAR should not be used - consider other model

3. Does the model prediction take into account relevant substance properties (water solubility, log K_{ow}, volatility, degradability)

   YES – Go to 3
   NO – (Q)SAR should not be used - consider other model

3. Are reliable estimates available from more than one (Q)SAR model?

   YES – use lowest predicted (Q)SAR endpoint in RA or qualifier for testing (if confirmed by WoE)
   NO – use single value as qualifier for testing if confirmed by WoE

Metabolites and Degradation Products

Criteria for definition of the residue for Risk assessment:

- individual components that at any time account for >10% of the amount of a.s. added
- individual components which in at least sequential measurements account for >5% of the amount of a.s. added
- individual components (>5%) for which, at the end of the study, the maximum of formation has not been reached
- other individual components present (if possible)

BUT – RA may be needed for residues <5% if indications that

- intrinsic properties comparable to parent
- high reactivity (eg mutagenic)
- endocrine disrupting properties
- unacceptable toxicological properties

➡ "Potentially relevant metabolites": need PEC and ecotox information

Toxophoric moiety

Substances with specific mode of toxicity have a structural feature responsible for the toxic property: TOXOPHORE

Transformation products of may retain or lose the toxophore

Sinclair and Boxall (2003)
70% of transformation products show similar/lower toxicity to parent
30% more toxic than parent
4% more than order of magnitude more toxic

Transformation products that are
- **more hydrophobic**
- **lack pesticidal activity of parent**

unlikely to be more toxic than parent

Sinclair CJ and Boxall ABA, 2003. Assessing the ecotoxicity of pesticide transformation products Environ. Sci. and Technol. 37, 4617–4625.

Risk assessment scheme for metabolites

Metabolites shown to have lost toxophore

– approximation of toxicity: compare exposure estimates to RAC_{parent} for most sensitive endpoint in relevant compartment

If fails – consider non-testing methods to further assess toxicity

Metabolites where toxophore is assumed to be retained – testing required

1) test in most sensitive taxonomic group wrt parent compound
2) >10x less sensitive than parent: assume toxophore lost
   - go to non-testing methods
3) Other Tier 1 studies where indicated:
   eg sediment-dwelling organisms
   chronic testing (guided by acute data, MOA)
   endocrine disrupting properties
   BCF

Combinations of a.s. in formulations

"..any information on potentially unacceptable effects of the PPP…as well as known and expected cumulative and synergistic effects"

(Comm Reg (EU) 284/2013)

AGD proposal reflects:

- typical availability of data for PPP and a.s.
- recent scientific opinions of the Commission
- existing approaches from regulatory authorities of several MS
- aim to improve mixture RA without increasing testing

Mixture toxicity

Measured:

Acute toxicity of formulation required for most sensitive taxonomic group (wrt a.s.)

Chronic testing required when formulation >10 x more toxic than a.s.

Calculated:

Concentration addition recognised as useful model for predicting mixture toxicity in PPPs

Not expected to be overly conservative (cp Independent action) at low exposure levels expected

Lower data demands for prediction than IA

Can be used to elucidate contribution of co-formulants to formulation toxicity

Model Deviation Ratio

$$\mathrm{ECx}_{mix-CA} = \left(\sum_{i=1}^{n} \frac{\mathrm{p}_i}{\mathrm{ECx}_i} \right)^{-1}$$

$$MDR = \frac{ECx_{mix-CA}\ (\text{calculated mixture toxicity})}{ECx_{PPP}\ (\text{measured mixture toxicity})}$$

MDR < 0.2 CA model overestimates formulation toxicity:

5 < MDR CA model underestimates formulation toxicity

0.2 < MDR < 5 CA use measured mixture toxicity ($EC_{x,ppp}$)

RA with measured mixture toxicity

$$ETR = PEC_{mix}/EC_{x,PPP}$$

But is the measured mixture (ie proportion a.s.) the same as the PEC mixture?
Hard to compare toxicity directly:

Compare calculated toxicity of PPP mixture vs PEC mixture

If $0.8 < EC_{x,PPP}/EC_{xmix\text{-}CA(PEC\ mix)} < 1.2$

→ use $EC_{x,PPP}$

Summary

- non-testing methods becoming available to support effects assessment
- Encouraged to help with minimization of testing
- Variety of (Q)SARs to predict toxicity – guidance available on assessing validity and applicability
- RA of metabolites may be aided by non-testing methods where toxophore is lost
- Concentration addition useful method for investigating toxicity of mixtures (>1 a.s., co-formulants) in PPPs