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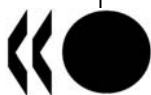
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JOINT MEETING OF THE CHEMICALS COMMITTEE AND  
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

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Number 101**

**REPORT OF THE WORKSHOP ON STRUCTURAL ALERTS FOR THE OECD  
(Q)SAR APPLICATION TOOLBOX**

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**No. 101**

**Report of the Workshop on Structural Alerts for the OECD (Q)SAR  
Application Toolbox**

**IOMC**

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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No. 84, *Report on the Workshop on the Application of the GHS Classification Criteria to HPV Chemicals, 5-6 July Bern Switzerland (2007)*

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No.90, *Background Review Document on the Rodent Hershberger Bioassay (2008)*

No.91, *Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3) (2008)*

No.92, *Report of the Validation Peer Review for the Amphibian Metamorphosis Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-Up of this Report (2008)*

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## **FOREWORD**

This document is a report of the Workshop on Structural Alerts for the OECD (Q)SAR Application Toolbox which was held on 15-16 May 2008 in Utrecht, the Netherlands. The workshop was agreed to be held at the 41st OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in June 2007.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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## REPORT OF THE WORKSHOP ON STRUCTURAL ALERTS FOR THE OECD (Q)SAR APPLICATION TOOLBOX, 15-16 MAY 2008, UTRECHT, THE NETHERLANDS

### BACKGROUND

1. The proof-of-concept version of the *OECD (Q)SAR Application Toolbox* (Toolbox) was publicly released in April 2008 (see [www.oecd.org/env/existingchemicals/qsar](http://www.oecd.org/env/existingchemicals/qsar)). It already contains, among other tools, a number of structural alerts relevant for potentially identifying specific hazards or for grouping (categorising)<sup>1</sup> chemicals. During the second phase of the development of the Toolbox which is expected to start in late 2008, a more extensive set of structural alerts will be integrated into the Toolbox.

2. In June 2007, the 41st OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to hold a *Workshop on Structural Alerts for the OECD (Q)SAR Application Toolbox* with the aim of identifying structural alerts which are useful to member countries in their chemical review activities and which should be implemented into the (Q)SAR Application Toolbox.

### WORKSHOP

3. The workshop was held on 15-16 May 2008 in Utrecht hosted by the Netherlands. The agenda is outlined in [Annex 1](#).

4. The workshop was attended by experts nominated by [Austria](#), [Czech Republic](#), [Denmark](#), [Germany](#), [Italy](#), [Japan](#), [Korea](#), [the Netherlands](#), [Spain](#), [Sweden](#), [Switzerland](#), [the United States](#), [the European Commission](#), [BIAC](#), [ICAPO](#), invited experts and the OECD Secretariat. The list of the participants is attached to this document as [Annex 4](#). The workshop was chaired by the OECD Secretariat.

#### Opening (Agenda 1)

5. Dick Sijm (RIVM) welcomed the participants on behalf of the Netherlands.

#### Scope and Objectives (Agenda Items 2 and 3)

6. The Secretariat presented the scope and objectives of the workshop for setting the scene. The stated scope of the Workshop was “to examine the current status of structural alerts (SAs)<sup>2</sup> as they relate to environmental fate, ecotoxicology and toxicology endpoints for organic chemicals”. The objectives of the Workshop were:

- To prioritise the incorporation of additional structural alerts in the Toolbox.
- To determine which structural alerts can be added in the future (short and medium term).

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<sup>1</sup> Also see: OECD (2007), *Guidance on Grouping of Chemicals*, Series on Testing and Assessment, No. 80, OECD, Paris, [http://appli1.oecd.org/olis/2007doc.nsf/linkto/env-jm-mono\(2007\)28](http://appli1.oecd.org/olis/2007doc.nsf/linkto/env-jm-mono(2007)28).

<sup>2</sup> In the context of the Workshop, there was no formal definition of a structural alert (SA). In general, a SA can be considered to be part of a molecule that may be associated with a toxicological effect. For example, for protein-binding-related toxicity mechanisms a SA can be thought of as a well defined molecular sub-structure or fragment.

- To stimulate further work on structural alerts.

7. To take the Toolbox forward into Phase 2, it has been agreed to examine the current status of structural alerts with the express purpose of identifying alerts which could assist in forming categories or sub-categories for data gap filling. This work will increase confidence in predictions made with the help of the Toolbox.

### ***Importance of Structural Alert-Based Categories***

8. The Secretariat indicated that the importance of SAs in the Toolbox is to allow for the formation of toxicologically meaningful categories, for instance based on intrinsic chemical reactivity. Such a category means that all the chemicals falling within it can be assessed when only a few members are tested. This enables transparent and defensible categories to be formed.

9. Currently the Toolbox only contains a relatively small number of SAs. Incorporation of new SAs is seen as being essential to add new functionalities to the Toolbox. The better the SAs, the better and more precise the category.

10. It is important to note that in the Toolbox alerts are not be used to predict activities or properties. Rather, the alerts are used solely to group chemicals to allow for read-across.

### **Structural Alerts Currently Used for Assessment Work (Agenda Item 4)**

11. A series of presentations was made by invited experts identifying A) SAs, which they are currently using for their assessment work, B) how those alerts are used, and C) a summary of the scientific background for those alerts. The abstracts of the presentations are attached as Annex 3 to this document and the main points from these presentations are summarized below.

### ***Mutagenicity and Carcinogenicity***

12. SAs for mutagenicity and carcinogenicity were reviewed by Aldo Benigni (Istitute Superiore di Sanita, Italy). These are seen as highly relevant for regulatory use. A number of SAs have been compiled and are now implemented in the ToxTree software (freely available from the website of the Joint Research Centre (JRC) of the European Commission [<http://ecb.jrc.it/qsar/>]). Most alerts are for genotoxic carcinogenicity but also a small number of alerts for non-genotoxic carcinogens are included. It was concluded that the current Toolbox alerts only included those for genotoxicity. Other capabilities for predicting carcinogenicity, in particular alerts for 'modulating' (either decreasing or increasing) carcinogenicity, include the OncoLogic software from the U.S. Environmental Protection Agency (EPA) [<http://www.epa.gov/oppt/newchems/tools/oncologic.htm>]. There was general agreement that modulating factors should be further investigated in the Phase 2 of the Toolbox.

### ***Eye Irritation and Corrosion***

13. Matthias Herzler (Federal Institute for Risk Assessment (BfR), Germany) discussed the BfR rules for eye irritation and corrosion. These are seen as of high regulatory significance. The BfR rules are based on physico-chemical properties as well as more classic structural alerts. The rules are available in the freely available ToxTree software [<http://ecb.jrc.it/qsar/>].

### ***Skin Irritation and Corrosion***

14. Etje Hulzebos (RIVM, the Netherlands) discussed the BfR rules for skin irritation and corrosion. This is an endpoint of high regulatory significance. The BfR rules are based on physico-chemical

properties as well as on more classic structural alerts. The rules are available in the freely available ToxTree software [<http://ecb.jrc.it/qsar/>].

### ***Acute Mammalian Toxicity***

15. SAs for acute mammalian toxicity were discussed by Harrie Buist (TNO). This is of a high regulatory significance. Currently the *in vitro* – *in vivo* correlations are not good enough. The difficulty of these extrapolations are well known, particularly as a result of the complexity of the mammalian systems (i.e. toxicokinetics of uptake, metabolism) as compared to cytotoxicity. This is an area where research is required in the longer term and databases need to be generated before SAs can be developed.

### ***Acute Aquatic Toxicity – Excess Toxicity***

16. Gerrit Schüürmann (Hermholtz Centre for Environmental Research (UFZ)) outlined the SAs, modes and mechanisms for acute aquatic toxicity and in particular highlighting the species dependency. There are well established mechanisms of excess toxicity already captured in Phase 1 of the Toolbox and the new work could be used to refine some of them.

### ***Aquatic Effects – Acute Toxicity, Biodegradation and Bioaccumulation***

17. Tala Henry (U.S. EPA) addressed issues relating to SAs firstly for acute aquatic toxicity and then for biodegradation and bioaccumulation. Updates of ASsessment Tools for the Evaluation of Risk (ASTER) [[http://www.epa.gov/med/Prods\\_Pubs/aster.htm](http://www.epa.gov/med/Prods_Pubs/aster.htm)] may improve category formation. The structural fragments in ASTER need to be compared to what is currently included in the Toolbox. The 44 class assignment functionalities from Ecological Structure Activity Relationships (ECOSAR (v0.99h)) [<http://www.epa.gov/oppt/newchems/tools/21ecosar.htm>] are currently available in the Toolbox. The new version of ECOSAR (v1.00) due to be released in mid-2008 will have a 130 classification scheme for aquatic toxicity. The inclusion of this new version will dramatically enhance the capability of the Toolbox to form meaningful categories.

18. The U.S. EPA BioWin software (included in Estimation Program Interface (EPI) Suite: <http://www.epa.gov/oppt/exposure/pubs/episuite.htm>) contains fragments for biodegradation. In addition ASTER contains structural features for degradable and non-degradable features. The inclusion of those fragments could improve the functionalities of the Toolbox.

19. Most bioconcentration prediction is currently based on log Kow. There are many attempts ongoing to refine the bioaccumulation field, most of which are not based directly on (Q)SAR.

### **On-Going Developments of Structural Alerts (Agenda Item 5)**

20. A series of presentations were made concerning on-going activities for developing new SAs. These presentations are summarized by endpoint or effect. The abstracts of the presentations are attached as Annex 3 to this document.

### ***Chemical Reactivity Relating to Fate***

21. Efforts in DuPont to describe features associated with chemical reactivity were described by Phil Lee (DuPont Haskell Global Centers for Health and Environmental Sciences). These were put in the context of degradation, both abiotic and biotic. Rather than alerts, these were termed as being structural profiles. Reactivity is used as part of a workflow to develop new compounds. Three different approaches are used to develop structural reactivity profiles. Much of this information is likely to remain proprietary.



22. Johann Gasteiger (Molecular Networks GmbH) described the reactivity fate tools being developed in his company under the contract with the JRC: START (Structural Alerts for Reactivity in Toxtree) and CRAFT (Chemical Reactivity And Fate Tool). Current JRC-funded projects include the development of a rule-based system for chemical reactivity relating to environmental fate and the development of a prototype software to predict the fate of chemicals.

#### ***Biotransformation in Aquatic Biota: Trans-Generic Biotransformation Potential***

23. Alistair Boxall (Central Science Laboratory, the United Kingdom) reviewed a CEFIC-LRI<sup>3</sup> project titled BiotS to identifying the trans-generic biotransformation potential of chemicals in the environment. Phase 1 of this project reviewed data on the biotransformation of chemicals in a variety of taxa and / or trophic levels for environmental species (mainly fish and invertebrates) with the construction of a database on the biotransformation of chemicals (in the environment). Data were collected for 85 biotransformation pathways for 310 metabolic steps for a total of 305 chemicals. Fragments associated with biotransformation were identified. The project is being expanded to a wider range of species in a new project. These new tools will ultimately be made freely available from CEFIC-LRI.

#### ***Estrogen Receptor Binding***

24. A chemical sub-class approach to form a decision support system for estrogen receptor (ER) binding was presented by Patricia Schmieder (U.S. EPA). The software developed at the US EPA should be freely available, within two years with possibilities of being placed within the Toolbox. In the mean time qualitative SAs for estrogen binding and ER gene expression are available and can be coded into the Toolbox rather easily.

#### ***Protein Binding Potency***

25. Terry Schultz (OECD Secretariat) described SAs for protein binding potency, with particular reference to the Toolbox. Currently qualitative SAs for 38 different mechanistic categories are already encoded in the Toolbox. These require further development to provide sub-categories for the express purpose of quantification.

#### ***Moving SAs into Rules (Alert-based Toxicity Prediction)***

26. Philip Judson (Lhasa Limited) discussed how SAs could be moved forward. He proposed that a system of rules is required to interpret the alert. The original alerts from the first version of DEREK [<http://www.lhasalimited.org/>] could be contributed to the Toolbox. He also suggested that Lhasa would be prepared to assist in the development of rules from alerts.

### **Conclusions and Recommendations (Agenda Items 6 and 7)**

27. After the series of presentations at Agenda Items 4 and 5, the meeting reviewed presentations and discussed priorities and recommendations to the Toolbox development. The meeting recognised that SAs are at the heart of the Toolbox and its development in Phase 2. There is considerable scope in terms of existing SAs that could be incorporated in the Toolbox in the near term and a number of on-going projects could also provide information before the end of Phase 2. The implementation of these SAs has been prioritized (see [Annex 2](#)).

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<sup>3</sup> CEFIC: Conseil Européen de l'Industrie Chimique / European Chemical Industry Council ; LRI: Long-Range Research Initiative

28. Agreed conclusions and recommendations for each area are attached as Annex 2.
29. The meeting also agreed upon other recommendations on general issues as follows:
- Continue efforts to gather alerts for grouping of chemicals into toxicologically meaningful categories;
  - For all sets of SAs in the Toolbox, develop documentation according to the QSAR Model Reporting Format (QMRFs)<sup>4</sup> as much as possible;
  - Encourage independent quality check of alerts and identify divergent interpretations.

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<sup>4</sup> See <http://ecb.jrc.it/qsar/> (direct link to QMRF: <http://ecb.jrc.it/qsar/qsar-tools/index.php?c=QRF>).

Also see Annex C to OECD (2007), *Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models*, Series on Testing and Assessment, No. 69, OECD, Paris, [http://apli1.oecd.org/olis/2007doc.nsf/linkto/env-jm-mono\(2007\)2](http://apli1.oecd.org/olis/2007doc.nsf/linkto/env-jm-mono(2007)2)

## ANNEX 1: AGENDA

<b>DAY 1: Thursday, 15 May 2008</b>		
09h00	<b>1</b>	<b>Opening</b> (10 min)
		The meeting will be opened by the Secretariat. RIVM will deliver welcoming remarks on behalf of the host country, the Netherlands. The participants will briefly introduce themselves. The Secretariat will explain housekeeping items.
09h10	<b>2</b>	<b>Scope and objectives</b> (10 min)
		The Secretariat will present the scope and objectives of the workshop, as well as the goal to be achieved at the end of the workshop.
09h20	<b>3</b>	<b>Current status of the use of structural alerts in the Toolbox</b> (30 min)
		The Secretariat will make a presentation on the first version of the OECD (Q)SAR Application Toolbox which was released in March 2008, focusing on the current status of the use of structural alerts in the Toolbox for potentially identifying specific hazards or for grouping (categorizing) chemicals. The latest plan for the second phase of the Toolbox development would be also presented, if possible.
09h50	<b>4</b>	<b>Structural alerts currently used for assessment work</b>
		A series of presentations will be made by member countries and stakeholders, identifying (i) structural alerts which they are currently using for their assessment work, (ii) how those alerts are used, as well as (iii) a summary of the scientific background [45 min for each presentation including questions.] <b>The participants will be invited to take note of the presentations, to provide comments as appropriate, and to consider how these structural alerts could relate to the Toolbox.</b>  <i>Part 1: Health</i> a) 09h50-10h35: Mutagenicity and carcinogenicity (Aldo Benigni, Istituto Superiore di Sanita, Italy) <b>[Coffee break: 10h35-10h50]</b> b) 10h50-11h35: Eye Irritation/Corrosion (Matthias Herzler, BfR, Germany) c) 11h35-12h20: Skin irritation/Corrosion (Etje Hulzebos, RIVM, the Netherlands) <b>[Lunch break: 12h20-14h00]</b> <i>Part 1: Health (cont.)</i> d) 14h00-14h45: Acute mammalian toxicity (Harrie Buist, TNO, the Netherlands)  <i>Part 2: Ecotoxicity</i>

		e) 14h45-15h30: Aquatic toxicity (Gerrit Schüürmann, Helmholtz Centre for Environmental Research (UFZ))  <i>Part 3: Fate</i>  f) 15h30-16h15: Aquatic toxicity, biodegradation and bioaccumulation (Tala Henry, U.S.EPA)
16h15		<b>Coffee break (15min)</b>
16h30	<b>5</b>	<b>On-going developments of structural alerts</b>
		A series of presentations will be made by member countries and stakeholders concerning on-going activities for developing structural alerts for regulatory purposes. [45 min for each presentation including questions and answers.]  <b>The participants will be invited to take note of the presentations, to provide comments as appropriate, and to consider how these activities could relate to the Toolbox.</b>  a) 16h30-17h15: Degradation: abiotic (hydrolytic, photolytic) and biotic (soil, plant, fish and animal) (Phil Lee, DuPont)  b) 17h15-18h00: Reactivity fate tool (Johann Gasteiger, Molecular Networks GmbH)
18h00		<i>Workshop adjourns for the day</i>

<b>DAY 2: Friday, 15 May 2008</b>		
08h30	<b>5</b>	<b>On-going developments of structural alerts [cont.]</b>
		c) 08h30-09h15: Identifying the Trans-Generic Biotransformation Potential (Alistair Boxall, Central Science Laboratory, United Kingdom)  d) 09h15-10h00: Receptor binding activity (Patricia Schmieder, U.S.EPA)  <b>[Coffee break: 10h00-10h15]</b>  e) 10h15-11h00: Protein binding potency (Terry Schultz, OECD Secretariat)  f) 11h00-11h45: Alert-based toxicity prediction (Philip Judson, Lhasa Limited, United Kingdom)
11h45		<b>Coffee break (15min)</b>
12h00	<b>6</b>	<b>Priorities for Inclusion to the Toolbox (60 min)</b>
		Given the presentations from Items 4 and 5 of the agenda, this session will serve to make recommendations (or set priorities) as to which structural alerts should be included into the (Q)SAR Application Toolbox.  <b>The participants will be invited to discuss priorities for inclusion into the Toolbox, as well as a time line and possible resources.</b>
13h00	<b>7</b>	<b>Conclusion (30min)</b>
		Following the discussion at preceding agenda items, the participants will be invited to confirm and agree on the conclusion of the workshop.
13h30		<i>Workshop adjourns</i>

## ANNEX 2: CONCLUSIONS AND RECOMMENDATIONS

## Agenda Item 4: Structural Alerts Currently Used for Assessment Work

<b>Mutagenicity and carcinogenicity</b> [Aldo Benigni, Istituto Superiore di Sanita, Italy]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Yes
• Availability of documentation	Yes
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	High
• Comments/recommendations	Explore how to integrate modulating factors
<b>Eye irritation/corrosion</b> [Matthias Herzler, BfR, Germany]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Yes
• Availability of documentation	Yes
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	High
• Comments/recommendations:	Explore how to integrate experimental results, recheck alerts. In phase 2 explore which tools to use to estimate physico-chemical (PC) properties, improve mechanistic understanding, further explore applicable domain (AD).
<b>Skin irritation/corrosion</b> [Etje Hulzebos, RIVM, the Netherlands]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Yes
• Availability of documentation	Yes
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	High
• Comments/recommendations:	Explore how to integrate experimental results, recheck alerts. In phase 2 explore which tools to use to estimate PC properties, improve mechanistic understanding, further explore AD.

<b>Acute mammalian toxicity</b> [Harrie Buist, TNO]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Under development
• Availability of documentation	Under development
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	Low
• Comments/recommendations:	Integrate databases first.
<b>Aquatic toxicity: Estimation of excess toxicity (UFZ)</b> [Gerrit Schüürmann, Hermholtz Centre for Environmental Research (UFZ)]	
• Relevance for regulatory decisions	High
• Availability of rule-base	About to be published
• Availability of documentation	About to be published
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	Medium (many categorisation mechanisms already in Toolbox)
• Comments/recommendations:	Highlight the species dependency
<b>Aquatic toxicity: ASTER (U.S. EPA)</b> [Tala Henry, U.S. EPA]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Yes
• Availability of documentation	Yes
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	Medium (many categorisation mechanisms already in Toolbox)
• Comments/recommendations:	Candidate to refine the OASIS acute toxicity MoA in the Toolbox
<b>Aquatic toxicity: ECOSAR 1.00 (U.S. EPA)</b> [Tala Henry, U.S. EPA]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Yes
• Availability of documentation	Yes (partially confidential)
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	High
• Comments/recommendation:	To replace ECOSAR 0.99 in the first version of the Toolbox

**Agenda Item 5: On-going developments of structural alerts**

<b>Biotic and abiotic reactivity</b> [Phil Lee, DuPont; Johann Gasteiger, Molecular Networks GmbH]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Under development or proprietary
• Availability of documentation	Under development or proprietary
• Availability of experimental data	Yes and more under development
• Priority for inclusion into the Toolbox	Medium
• Comments/recommendations:	Wait for results, start with ASTER alerts
<b>Biotransformation in aquatic biota</b> [Alistair Boxall, Central Science Laboratory]	
• Relevance for regulatory decisions	High
• Availability of rule-base	About to be published
• Availability of documentation	About to be published
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	High
• Comments/recommendations:	Wait for phase 2
<b>Estrogen receptor binding activity</b> [Patricia Schmieder, U.S. EPA]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Yes
• Availability of documentation	Yes
• Availability of experimental data	Yes
• Priority for inclusion into the Toolbox	High
• Comments/recommendations:	Await further peer-review, start with qualitative rules
<b>Protein binding potency</b> [Terry Schultz, OECD Secretariat]	
• Relevance for regulatory decisions	High
• Availability of rule-base	Under development
• Availability of documentation	Under development
• Availability of experimental data	Under development
• Priority for inclusion into the Toolbox	High
• Comments/recommendations:	Wait for phase 2

### ANNEX 3: ABSTRACTS OF PRESENTATIONS

#### Agenda Item 4: Structural alerts currently used for assessment work

##### *Mutagenicity and carcinogenicity*

- Structure-Alerts for mutagenicity and carcinogenicity (Aldo Benigni, Istituto Superiore di Sanita, Italy) ... page 13

##### *Eye irritation/corrosion*

- The BfR Rules/Alerts for eye irritation, serious eye damage and corrosion (Matthias Herzler, BfR, Germany) ... page 16

##### *Skin irritation/corrosion*

- (Q)SARs for predicting the absence and presence of skin irritation for the OECD Toolbox (Etje Hulzebos, RIVM, the Netherlands) ... page 20

##### *Acute mammalian toxicity*

- A QSAR for worst case estimates of acute toxicity of chemically reactive compounds (Harrie Buist, TNO) ... page 24

##### *Aquatic toxicity (ASTER, ECOSAR 1.00); Biodegradation (BioWin, ASTER) and Bioaccumulation*

- Structure alerts for aquatic toxicity, biodegradation and bioaccumulation (Tala Henry, U.S. EPA) ... page 29

#### Agenda Item 5: On-going developments of structural alerts

##### *Abiotic and biotic reactivity*

- Development of metabolic structural alerts for persistence, bioaccumulation and toxicity (PBT) hazard characterization (Phil Lee, DuPont Haskell Global Centers for Health and Environmental Sciences) ... page 33
- Structural alerts and chemical reactivity in risk assessment (Johann Gasteiger, Molecular Networks GmbH) ... page 34



*Trans-generic biotransformation potential*

- Biotransformation susceptibility of chemicals in aquatic organisms – BiotS (Alistair Boxall, Central Science Laboratory) ... page 36

*Endocrine receptor binding*

- A decision support system for prioritizing ER binding potential within defined regulatory inventories: a chemical sub-class approach (Patricia Schmieder, U.S. EPA) ... page 37

*Protein binding*

- Structural alerts for protein binding: sub-categories & adding the dimension of potency (Terry Schultz, OECD Secretariat) ... page 40

*Alert based toxicity prediction*

- Alert-based toxicity prediction at Lhasa Limited (Philip Judson, Lhasa Limited) ... page 42

## Structure-Alerts for mutagenicity and carcinogenicity

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Historically, the electrophilic theory of chemical carcinogenesis developed by James and Elizabeth Miller (1, 2) enabled the activity of the large majority of animal carcinogens known by the 1970's to be tentatively rationalized. The evidence found led to the suggestion "that most, if not all, chemical carcinogens either are, or are converted *in vivo* to, reactive electrophilic derivatives which combine with nucleophilic groups in crucial tissue components, such as nucleic acids and proteins" (2).

Based on their seminal work, Bruce Ames created a series of genetically-engineered *Salmonella typhimurium* bacterial strains, each strain being specifically sensitive to a class of chemical carcinogens (e.g., alkylating, intercalating, etc.) (3). Since most of the known carcinogens at that time acted through genotoxic mechanisms, the activity of chemicals as mutagens to *Salmonella* almost always seems plausible within the context of the Millers' hypothesis (4).

After a number of decades, the hypothesis of the electrophilic reactivity of (many) chemical carcinogens maintains its validity, and has been incorporated into a more general theory on the chemical carcinogens. From the point of view of mechanism of action, carcinogens are classified into: a) genotoxic carcinogens, which cause damage directly to DNA. --many known mutagens are in this category, and often mutation is one of the first steps in the development of cancer (5); and b) epigenetic carcinogens that do not bind covalently to DNA, do not directly cause DNA damage, and are usually negative in the standard mutagenicity assays (6).

John Ashby translated the mechanistic knowledge on chemical carcinogenesis into a list of Structural Alerts (SA) (7) (4). The SAs for carcinogenicity are defined as molecular functional groups or substructures that are linked to the carcinogenic activity of the chemicals. Thus, they identify the major chemical classes potentially able to cause cancer. Since the attack to, and the modification of DNA is the main step in the mechanism of action of many carcinogens (i.e., the so-called genotoxic carcinogens), the SAs relative to such classes of carcinogens are also valid for the mutagenicity endpoint.

The SAs hold a special place in predictive toxicology. The knowledge on the action mechanisms as exemplified by the SAs is routinely used in Structure-Activity Relationship (SAR) assessment in the regulatory context (see, for example, (8)). In addition, the SAs are at the basis of popular commercial (e.g., DEREK, by Lhasa Ltd. ) and noncommercial software systems (e.g., Oncologic, by US Environmental Protection Agency <http://www.epa.gov/oppt/newchemicals/tools/oncologic.htm>).

Following Ashby's work, other lists of SAs have been reported ((9, 10, 11). For reviews, see (12, 13). Recently, the ability of the SAs to uncover carcinogens/mutagens in large databases of chemicals experimentally tested has been compared in the framework of a collaboration between the European Chemicals Bureau (ECB) and the Istituto Superiore di Sanita' (ISS) (14) (see also the report (15). Overall, the four SA models did not differ to a large extent in their performance. In databases including chemicals from diverse chemical classes, the SA models appear to agree around 65% with rodent carcinogenicity data, and 75% with *Salmonella* mutagenicity data. As an exception, the Bailey' SAs exhibit lesser specificity (higher false positives) than the Ashby' SAs, without a comparable increase in sensitivity. In addition, the SA models do not work equally efficiently in the discrimination between active and inactive chemicals

within individual chemical classes: their poorer performance can be ascribed to the fact that the SA models considered lack sub-rules detailed enough as to be able to describe how each alert is modulated by the different molecular environments.

Within the above limits, the SAs have a unique role for: a) description of sets of chemicals; b) preliminary hazard characterization; c) formation of categories for e.g., regulatory purposes; d) generation of subsets of congeneric chemicals to be analyzed subsequently with QSAR methods; e) priority setting.

Following the survey performed by ECB and ISS, an updated list of SAs has been compiled, and implemented into a new module of the expert system Toxtree (v. 1.4) (<http://ecb.jrc.it/qsar/qsar-tools/>). The SAs included in this expert system are 33; out of them, 5 SAs refer to nongenotoxic mechanisms of action. The analysis shows that this revised list of SAs has increased sensitivity and accuracy in respect to the Ashby's alerts, at the cost of a diminished specificity. Thus an overall increase in performance is apparent. Details on the Toxtree module are in (16).

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## The BfR Rules/Alerts for Eye Irritation, Serious Eye Damage, and Corrosion

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### Eye Irritation/Serious Damage/Corrosion – (A) Defined Endpoint(s)

In the EU, two risk phrases ('R-phrases') are used to characterise irritant/corrosive substances according to the scores and reversibility/irreversibility of eye lesions observed in this test (if not derived from other available information): R36 ('irritating to eyes') and R41 ('risk of serious damage to eyes'). In addition, compounds causing full-thickness destruction of skin are termed 'corrosives' (R34 'causes burns' or R35 'causes severe burns') and for these substances labelling with R41 is implicit without actual performing an eye irritation test.

Investigations into the prevalence of irritants/corrosives among (New) Industrial Chemicals in the EU have demonstrated that about 75 % of all substances are non-irritant. As a consequence, existing information/*in vitro* tests/(Q)SARs that are able to positively predict eye irritation/corrosion will be inconclusive, while for irritants - and even more so for corrosives - prediction with sufficient certainty will often be possible. While an otherwise well-established integrated testing strategy (cf. OECD test guideline 405) has significantly reduced animal testing for eye irritation, it remains a paradox that the majority of remaining Draize tests will be performed on non-irritants. As a consequence, successful prediction of the ABSENCE of eye irritation/corrosion would be highly appreciated. Remarkably, the EU REACH legislation appears to be the first regulatory framework to officially endorse the use of (Q)SARs for such 'negative prediction', if sufficiently validated.

### The BfR Rules/Alerts - Chemical Space and Algorithms Used

From 1982-2002, Gerner et al. at the BfR (formerly BGA, BgVV) compiled a database of phys.-chem. property, acute toxicity, local irritation/corrosion, and sensitisation data of some 1600 substances from the EU New Chemicals Notification program (database 'ESTOFF', cf. Gerner 2000, Herzler 2006). All data were peer-reviewed for validity and quality by using identical assessment criteria. ESTOFF contains 102 (6.3 %), 257 (15.8 %), and 77 (4.7 %) chemicals classified/labelled as R34/35, R41, and R36, respectively, as well as 1191 (73 %) compounds not requiring classification with respect to eye lesions. Based on the data available at the respective time (1358 compounds in 2002), two types of prediction tools were published (Gerner 2000) and later updated (Gerner 2005, Hulzebos 2005, Walker 2004):

- a) 31 physico-chemical exclusion rules for predicting the ABSENCE of eye irritation/corrosion potential (7 rules for all compounds, 24 rules for subclasses of different chemical composition). These rules follow a straight-forward, unambiguous

'**IF** (phys.-chem. property limit value is exceeded) **THEN NOT** (R36, R41, and/or R34/35)'

logic. Following empirical analysis of the distribution of the training set over the descriptors (molecular weight/m.w., melting point/m.p., log P, aqueous solubility/a.s. and lipid solubility/l.s.), limit values were established to exclude the respective 100 % percentiles of R36, R41, and/or R34/35 chemicals.

- b) 27 structural alerts for positive prediction of eye irritation/corrosion potential. These take on the general form of reactive chemical substructures as published by other authors for a variety of toxicological endpoints (e.g. the Ashby/Tennant alerts for *S. typhimurium* mutagenicity) or contained in commercial software (such as DEREK).

### **Applicability Domain (AD) of the BfR Rules/Alerts**

A substance purity of  $\geq 95$  % and the absence of any reactive impurities are general pre-requisites for applying the BfR rules/alerts. Likewise, a tendency to form irritant/corrosive decomposition products, e.g. after hydrolysis, must be excluded. Lesser substance purity might be tolerable, if the impurity profile is characterised quantitatively and the presence of irritant/corrosive impurities can be ruled out. Inorganic substances are principally out of AD.

If all of these conditions are met, the AD of an individual phys.-chem. exclusion rule is characterised by the chemical class it is designed for (e.g. class CN = substances of composition  $C_xH_yO_zN_a$ ) and by the limit value itself, i. e. the rule is only applicable, if the limit value is exceeded for the compound in question. E.g. all substances with m.p.  $\leq 200$  °C are outside the AD of the rule 'IF (m.p.  $> 200^\circ\text{C}$ ) THEN NOT (skin corrosion R34 or R35)' and no statement on their eye irritation/corrosion potential may be derived from this rule.

Quoting (Netzeva 2005), the 'AD for an alert [...] can be defined simply in terms of the scope of the alert. If a chemical contains the alert, then it lies within the domain; if it does not contain the alert, then it lies outside the domain, in which case no conclusion for or against toxicity can be drawn.' In addition, the established phys.-chem. rules further limit the AD of any (BfR or other) alert for eye irritation from a mechanistic point of view (cf. next section).

#### **'A Mechanistic Interpretation, If Possible...'**

Corrosive substances, such as strong acids or bases, exert their effect by direct chemical reaction with and destruction of tissue. In contrast, for R36 or R41 compounds, the exact mechanisms of eye irritation on the molecular scale are only poorly understood. However, in general terms, a substance would a) have to be able to pass physiological barriers present in the eye (e.g. tear film, corneal multilayer structure etc.) in order to b) reach the place of interference with biological structures/processes leading to manifestation of relevant eye lesions.

As to a), the descriptors used in the phys.-chem. exclusion rules can be tentatively interpreted as being governed by similar molecular properties and intra-/intermolecular interaction forces relevant for transport/partitioning processes through membranes or into liquid layers of different composition. In this general picture, the structural alerts would then point at specific chemical reactivity required for b).

### **Validation Results Obtained for the BfR Rules/Alerts**

Both internal and external validation of the BfR rules/alerts have been performed by different authors. (Gerner 2005) demonstrated that a high percentage of non-irritants fell into the AD of one or more phys.-chem. rules, thereby underlining their potential relevance. Both the training set and a test set of ca. 200 Industrial Chemicals were investigated (dataset of overall  $> 1600$  compounds containing 27 % irritants or corrosives). Aside from 10 chemicals, which turned out to be outside the AD (irritant decomposition products), no false negative predictions were obtained for any rule (average Negative Predictive Value, NPV = 100 %).

The same datasets were also validated by Tsakovska and co-workers (Tsakovska 2005 and 2007). They confirmed the principal suitability of the test set for validating the rules/alerts, the good agreement with requirements of the OECD validation principles and the high NPVs of the phys.-chem. rules. One of the descriptors (lipid solubility) turned out to be of limited/no value due to low availability of data.

For the structural alerts, high false negative prediction rates were reported, however, upon closer inspection of alleged misclassifications it turned out that the authors had interpreted the prediction results differently from the way intended by Gerner and co-workers. E.g. substances not classified for eye irritation/corrosion based on experimental results, but predicted as R36 or R41, were rated as misclassifications ignoring the fact that a structural alert can only indicate a potential for reactivity, while phys.-chem. factors might preclude the substance from reaching the target tissue and actually exerting a toxic effect. Furthermore, in interpreting correct/incorrect predictions, the authors did not sufficiently take into account the different basis for classifications with R41 and R34/35.

Finally, preliminary results from the application of the rules/alerts to substances included into annex I of Dir. 91/414/EEC (pesticide active ingredients) demonstrated a high relevance, in particular of the exclusion rules for corrosion. Of 21 chemicals (incl. 1 R36 and 2 R41 substances) 76 % fell into the AD of at least one rule. Of these, 19, 13, and 100 % were correctly predicted not to be irritant, causing serious eye damage, or being corrosive, respectively. Again, no false negative predictions were obtained

### Conclusions and Recommendations for Further Work

In general, the BfR rules/alerts comply well with the OECD principles for the validation of (Q)SARs. A high degree of relevance and very good predictivity of the phys.-chem. exclusion rules have been demonstrated for Industrial Chemicals during internal and external validation by different authors. Preliminary results also suggest considerable relevance to hazard assessment of pesticide active ingredients. For the structural alerts, in general good prediction results were obtained. However, alerts were not always found to be unambiguous. In contrast, the phys.-chem. exclusion rules are straightforward and easy to apply.

In summary, extensive and successful validation has been performed on the BfR rules/alerts, and detailed documentation is available

For integration of the BfR rules/alerts into the OECD Toolbox, perhaps critical re-evaluation of some of the validation results obtained so far should be considered. Furthermore, a more unambiguous definition of some of the structural alerts is needed. More/better guidance, *inter alia* on assessment of whether a chemical to be predicted really falls into the AD of a rule, alert, or their combination and on the interpretation of the respective prediction results should be elaborated. Finally, extending work on pesticides/biocides and towards other chemical spaces appears worthwhile. Application of multivariate statistical data mining and/or classification tools (e.g. DA, PCA) to the ESTOFF database might be rewarding

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**(Q)SARs for predicting the absence and presence of skin irritation for the OECD Toolbox**

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**Introduction**

Gerner et al. (2004) established physico-chemical cut off rules to predict the absence of EU Classification and Labelling for skin irritation. These rules were validated by Rorije and Hulzebos (2005). Hulzebos et al. (2005) established structural alerts for the prediction of the presence of skin irritation and corrosion. The goal of the present paper is to propose to include the rules, alerts and examples in the OECD Toolbox.

**Method**

For the physico-chemical cut off rules the table in the publication of Rorije and Hulzebos (2005) was cleaned up and the numbers of chemicals to which the rules apply were added from Gerner et al., 2004. These rules are divided into rules applicable to all substances (Class: All) and applicable to specific chemical subclasses that are defined according to the definitions given in Table 1. The seven physico-chemical parameters used for defining skin irritation rules and their abbreviations and units are given in Table 2.

The structural alerts of Hulzebos et al. (2005) were refined by Rorije et al. (unpublished data) and examples were added, which were collected from Annex I, the EU list for classified chemicals (EC, 2004)

Table 1. Definition of structural classes for which separate skin irritation exclusion rules have been defined.

Class	Structure contains only	Formula
C	Carbon (C), hydrogen (H) and oxygen (O) atoms	$C_xH_yO_z$
CN	C, H, O and Nitrogen (N) atoms	$C_xH_yO_zN_a$
CNHal	C, H, O, N and Halogen (Hal) atoms	$C_xH_yO_zN_aHal_b$
CNS	C, H, O, N and Sulphur (S) atoms	$C_xH_yO_zN_aS_b$
CHal	C, H, O and Halogen (Hal) atoms	$C_xH_yO_zHal$

Table 2. Physico-chemical parameters used for defining skin irritation rules, with their respective abbreviation and units used throughout this report.

Parameter	Abbreviation	Unit
Molecular Weight	MW	g/mol
Melting Point	m.p.	°C
Octanol Water partition coefficient	log Pow	-
Aqueous solubility	a.s.	g/kg
Surface tension	s.t.	mN/m
Lipid solubility	l.s.	g/kg
Vapour Pressure	v.p.	Pa

**Results**

In table 3 the set of rules are presented for the classes mentioned in table 2. The rules use a set of descriptors which need to be fulfilled to come to the result of absence of EU Classification. These set of

descriptors are given in the IF column. These descriptors are the class of chemical, the physico-chemical property, the direction of how the cut off value should be read (qualifier), the actual cut off value, and the units of this value. The result is presented in the THEN column and shows the absence of EU Classification for skin irritation and corrosion, R38 and R34/35 respectively. For the presented (Q)SARs the distinction between R34 and R35 was not made.

In the next column the number of chemicals, to which the rule applies, is given. In most cases the rule applies to all chemicals in that class. This minimised the number of false negatives. For exceptional cases a note was added. These exceptions can be generalised in a prerequisite that the rules may not apply to very strong (inorganic) reactives.

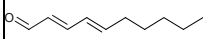
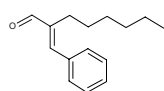
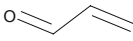
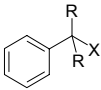
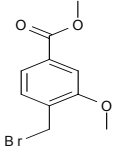
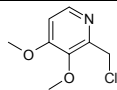
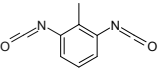
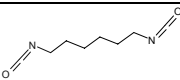
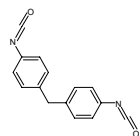
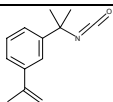

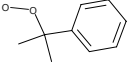

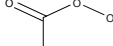
Table 3: Overview of physico-chemical exclusion rules for skin irritation/ corrosion established by Gerner et al.2004.

GROUP	IF Parameter	Qualifier	Value	Unit	THEN NOT	Number of chemicals to which the rule applies/total number of chemicals for this rule	note
All	m.p.	>	200	°C	R34, R35, or R38	291/297	1
All	l.s.	<	0.01	g/kg	R34 or R35	60/60	
All	log P <sub>ow</sub>	<	-3.1		R34, R35, or R38	56/56	
All	log P <sub>ow</sub>	>	9		R34, R35	Gerner et al., 2005	
C	m.w.	>	350	g/mol	R34 or R35	93/93	
C	m.p.	>	55	°C	R34, R35, or R38	128/130	2
C	a.s.	<	0.0001	g/mol	R34 or R35	Gerner et al., 2005	2
C	s.t.	>	62	mN/m	R34 or R35	94/95	3
C	v.p.	<	0.0001	Pa	R38	73/73	
CN	m.w.	>	290	g/mol	R34 or R35	338/338	
CN	m.w.	>	540	g/mol	R34, R35, or R38	86/86	
CN	m.p.	>	180	°C	R38	153/153	
CN	log P <sub>ow</sub>	>	4.5		R34 or R35	119/119	
CN	log P <sub>ow</sub>	>	5.5		R34, R35, or R38	85/85	
CN	a.s.	<	0.1	g/L	R34 or R35	280/280	
CN	a.s.	<	0.0001	g/L	R34, 35 or R38	104/104	
CN	a.s.	<	0.1	g/L	R34, R35	280/280	
CN	v.p.	<	0.001		R34 or R35	273/273	
CN	l.s.	<	0.4	g/kg	R34 or R35	56/56	
CNHal	m.w.	>	380	g/mol	R34, R35, or R38	99/99	
CNHal	log Pow	>	3.8		R34, R35, or R38	70/70	
CNHal	a.s.	<	0.1	g/L	R34 or R35	135/135	
CNHal	a.s.	<	0.001	g/L	R34, R35, or R38	78/78	
CNHal	l.s.	<	400	g/kg	R34 or R35	76/76	
CNHal	l.s.	<	4	g/kg	R34, R35, or R38	29/29	
CNS	m.w.	>	620	g/mol	R34 or R35	53/53	
CNS	m.p.	>	50	°C	R34 or R35	179/180	4
CNS	m.p.	>	120	°C	R34, R35, or R38	137/137	
CNS	log Pow	<	0.5		R38	96/96	
CNS	log Pow	<	-2		R34, R35, or R38	Gerner et al., 2005	
CNS	s.t.	>	62	mN/m	R34 or R35	92/92	
CHal	m.w.	>	280	g/mol	R34 or R35	59/59	
CHal	m.w.	>	370	g/mol	R34, R35, or R38	24/24	
CHal	m.p.	>	65	°C	R34 or R35	Gerner et al., 2005	
CHal	log Pow	>	4.5		R34 or R35	Gerner et al., 2005	

1. Six irritant/corrosive substances are organic salts which release strong inorganic acids or bases when getting in contact with aqueous substrates/organic media. 2. Two irritant/corrosive substances are organic salts which release strong inorganic acids or bases when getting in contact with aqueous substrates/organic media. 3. One corrosive substance is a skin defatting ether with high vapour pressure. 4. One corrosive substance is an organic Li salt which releases LiOH when getting into contact with aqueous substrates/organic media

In Table 4 four types of alerts are presented and specific examples have been added. These alerts indicate the presence of skin irritation or skin corrosion. For the examples given this presence of skin irritation or corrosion resulted in an EU Classification, R38 and R34/35, respectively (last column).

Table 4 Structural alerts for the presence of skin irritation or corrosion following EU Classification rules

Alert name	Alert description	Examples	EU Classification
Michael addition mechanism	$R-CH=CH-CHO$		R38
			R38
			R34
Aromatics with potential leaving group	 <p>X = -Cl, -Br, -I, -OSO<sub>2</sub>R  R = -H, -C-anything, not =O  the benzyl ring can be substituted,  the reactive carbon atom can not  be part of any ring system</p>		R38
			R38
(Di)Isocyanate	$O=C=N-R$ R can be anything		R38
			R38
			R38
			R34
(Hydro) peroxides	$R^1-O-O-H$ R= -H, -C, -P, -S → CORROSIVE $R^2-O-O-R^2$ R= -C, -P, -S → IRRITANT except:  → not irritant, but pH of resulting hydrolysis products (carboxylic acids) should be considered		R34
			R34
			R34

Substances, which act by a “Michael addition” mechanism are expected to do so at the conjugated C=C double bond. Skin irritation as well as skin corrosion can occur (table 4).

Substances, which react with proteins by “S<sub>N</sub>2 substitution” will do so at the site of the leaving group. For example, benzyl halides, undergo “S<sub>N</sub>2 reactions” with strong and weak nucleophiles and can cause skin irritation (table 4).

The (di)isocyanates are strong reactives in contact with water or organic material. This can result in skin irritation or corrosion.

The (hydro)peroxides are oxidisers. The reactivity of hydroperoxides will usually result in skin corrosion, while that of peroxides will more often result in skin irritation (EC, 2004).

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## A QSAR for worst case estimates of acute toxicity of chemically reactive compounds

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This summary is largely based on an article published in Toxicology Letters (Freidig *et al.*, 2007; doi:10.1016/j.toxlet.2007.03.008) and extended with some additional data generated in the research leading to this article, which have not been published before.

### 1. Introduction and summary of Materials & Methods

Future EU legislations enforce a fast hazard and risk assessment of thousands of existing chemicals. If conducted by means of present data requirements, this assessment will use a huge number of test animals and will be neither cost nor time effective. The purpose of the current research was to develop methods to increase the acceptability of *in vitro* data for classification and labelling regarding acute toxicity. For this purpose, a large existing database containing *in vitro* and *in vivo* data was analysed for correlations using the linear regression function of MS Excel. A detailed description of these data can be found in the publication of Halle (2003). For more than 300 compounds in the database, relations between *in vitro* cytotoxicity and rat or mouse intravenous and oral *in vivo* LD50 values were re-evaluated and the possibilities for definition of mechanism based chemical subclasses were investigated. For external validation we used a dataset compiled under the MEIC project (Clemedson and Ekwall, 1999, Clemedson *et al.*, 2000 and Ekwall, 1999) which consists of 50 chemicals. Compounds present in the external validation set were removed from the training data set. The reduced training data set contained 311 compounds, consisting of drugs, agrochemicals and industrial chemicals.

### 2. Results

#### 2.1. *In vitro*–*in vivo* correlations of compounds in training set

Correlations between the median *in vitro* cytotoxicity (log IC50 in mmol/L) and the *in vivo* log LD50 values (in mmol/kg) for the full training set are given in Table 1. Although this indicates a relation between *in vitro* and *in vivo* values, these correlations are considered not precise enough for a reliable prediction of acute toxicity for individual chemicals.

Table 1. Correlations of *in vitro* cytotoxicity (log IC50) and *in vivo* acute toxicity (log LD50)

Species and route	Full training set		Substances classified for irritation		Substances not classified for irritation	
	$r^2$	$n$	$r^2$	$n$	$r^2$	$n$
Rat oral	0.40	259	0.56	107	0.21	152
Rat iv	0.61	110	0.77	41	0.43	69
Mouse oral	0.44	248	0.63	88	0.29	160
Mouse iv	0.53	142	0.67	47	0.44	95

A close inspection of the compounds which were well described by the regression models revealed that many of these compounds contained chemically reactive groups, frequently included as structural alerts in SAR's on skin or eye irritation (Hulzebos *et al.*, 2003 and Hulzebos *et al.*, 2005, Walker *et al.*, 2004; see table 2). This observation led to the hypothesis that the acute toxicity of chemicals which are irritating can be well predicted using *in vitro* cytotoxicity data.

However, the correlation is weak and also restricting the prediction to the respective chemical domains of the structural alerts did not sufficiently improve it (data not shown). Therefore, we opted to use classification for irritation based on *in vivo* tests as a means to restrict the applicability domain of the prediction.

Table 2 Presence of structural alerts in well predicted and badly predicted chemicals (Observed number /expected number)

Structural alert (no. of cases)	Absolute log(deviation) <sup>5</sup>		
	low <sup>1</sup>	medium <sup>2</sup>	high <sup>3</sup>
acids (39)	1.0	1.0	1.0
phenols (33)	1.3	0.7	1.1
primary_aliphatic_amines (87)	1.0	1.0	0.9
secondary_aliphatic_amines (75)	1.0	1.1	0.8
tertiary_aliphatic_amines (46)	1.2	1.1	0.7
alkylalkanolamines (16)	1.2	1.2	0.5
sulfonic_acids (10)	1.4	0.3	1.6

<sup>1</sup> < log2; <sup>2</sup> between log 2 and log 5; <sup>3</sup> >log 5

## 2.2. Establishment of QSAR and chemical domain

The original training set was split in substances categorized for irritation and substances not categorized for irritation. The best correlations were found for the prediction of rodent toxicity data for iv dosing (see Table 1). This was to be expected, because the iv dosing route is generally to 100% bioavailability of the test compound, a worst case situation compared to the oral route. These iv data of irritating compounds were further screened for the presence of outliers assignable to a well definable subdomain. Thereby the sub class of anti-neoplastic drugs was identified and excluded from the chemical domain. Because the preferred test species for classification and labelling of acute oral toxicity is the rat (GHS, 2003), rat and mouse *in vivo* data were combined in a rodent data set by using rat data if available, and otherwise mouse data. For the iv data set in the applicability domain of irritating compounds not being anti-neoplastic agents, an excellent correlation across a wide range of toxic doses was observed (Fig. 2). The linear regression model for this correlation is given by Eq. (1). A cross validation, leaving out 20% of the data was performed as internal validation.

$$\log \text{LD50 (iv) [mmol/kg]} = 0.620(\pm 0.037) \log \text{IC50 [mmol/L]} + 0.002(\pm 0.069) \quad (1)$$

$$n = 49, r^2 = 0.854, \text{SEC} = 0.477, q^2_{\text{int}} = 0.835, F = 275.7^6$$

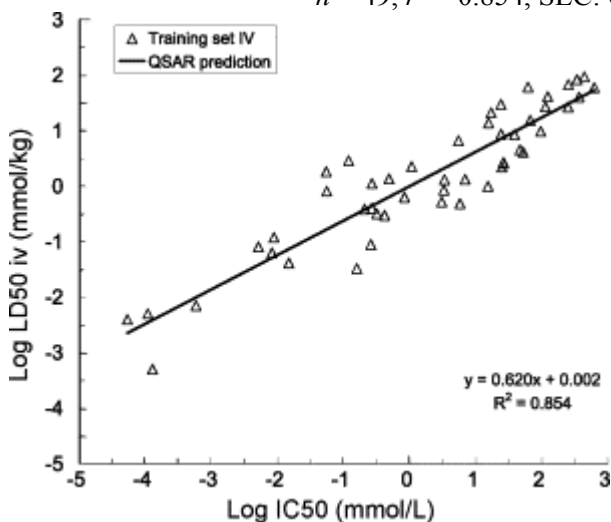


Fig. 1. Correlation between median *in vitro* toxicity and *in vivo* LD 50, obtained from rodents studies exposed intravenously, for 49 chemicals which are irritants but not anti-neoplastics.

<sup>5</sup> = absolute (log( IC<sub>50-in vitro</sub> / LC<sub>50 in-vivo</sub>))

<sup>6</sup> *n* is the number of compounds, *r*<sup>2</sup> the coefficient of determination, SEC the standard error of calibration (also known as standard error of the estimate, in the linear regression output), *F* the variance ratio (tests whether model has statistically significant predictive capability) and *q*<sup>2</sup><sub>int</sub> is obtained after internal validation.

### 2.3. Worst case estimates for acute toxicity classification

With the QSAR given as Eq. (1), acute oral toxicity in rodents was predicted based on *in vitro* cytotoxicity data. This QSAR gives a worst case prediction as the correlation is based on *in vivo* data and oral dosing can lead to a reduced bioavailability. This is illustrated in Fig. 2.

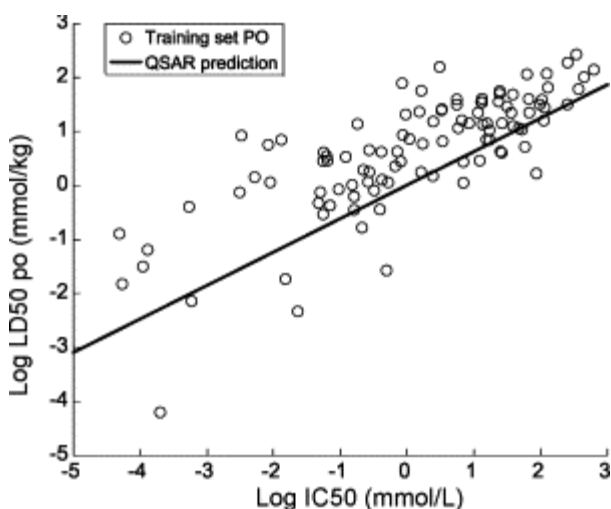


Fig. 2. Correlation between median *in vitro* toxicity and *in vivo* oral LD 50 for 106 chemicals from the training set. The worst case QSAR (EQ1) derived from *in vivo*  $LD_{50}$ 's is given as solid line.

### 2.4. Application of QSAR on validation set

The whole MEIC validation data set was split into 2 sub sets: 13 chemicals for which *in vivo* data was available and 21 chemicals for which oral data was available. The *in vivo* set was subsequently used as external validation for the worst case prediction model, which yielded the following statistics:  $q^2_{ext} = 0.710$ , SEP = 0.740, where  $q^2_{ext}$  is obtained after external validation using validation test set and SEP is the standard error of prediction.

### 2.5. Predict labelling and classification for acute toxicity

For the classification and labelling of compounds for oral acute toxicity an  $LD_{50}$  value in mg/kg bodyweight was used. We calculated  $LD_{50}$  values (mg/kg bodyweight) using Eq. (1) and the MW of the chemicals, and applied the resulting values to the classification scheme as proposed by the GHS. The predicted classification based on the estimated  $LD_{50}$  values was compared with the classification based on the experimental  $LD_{50}$  values (Table 3).

Table 3. Predictions of acute oral toxicity classification (in accordance with the GHS) using the QSAR for a worst case estimate

Predicted classification	Training set (n = 106)	Validation set (n = 21)
Correct class	39%	43%
Overprediction	57%	43%
Underprediction	4%	14%

Results showed that the approach of defining a worst case estimate clearly worked for both the training and the validation set. Only 4% and 14% of the chemicals were underestimated regarding their acute oral toxicity.

### 3. Discussion and conclusions

In this paper, we presented a case study for developing and testing a QSAR for a mammalian toxicological endpoint (acute toxicity) considering both its scientific validity and its application in regulatory context (classification and labelling).

For this QSAR, the applicability domain was defined using the classification of chemicals as irritating. Although the initial hypothesis was based on the overrepresentation of chemicals with structural alerts for irritation among the best predicted data, the classification data were based on *in vivo* experiments, which is a drawback of this applicability domain definition. However, there are numerous efforts to replace the *in vivo* tests for irritation by either *in vitro* tests or by a combination of *in vitro* tests and SAR's. Once these alternatives are established, they can also be used to define the applicability domain for the acute toxicity QSAR presented above. During the development of the QSAR, there was no indication for an upper or lower limit regarding toxicity. The only outlier class was defined again on a property (anti-neoplastic drug) and not on structural criteria.

The test set for iv data yielded a very strong correlation between *in vitro* cytotoxicity and *in vivo* iv LD<sub>50</sub> ( $r^2 = 0.85$ ) with low standard deviations and a high internal  $q^2$ . External validation with an independent data set, showed that: (i) the iv data was predicted with an external  $q^2$  of 0.71 and (ii) in the oral data, 3 out of 21 compounds were more toxic than predicted, despite that they fulfil the criteria of the applicability domain.

It is well known that irritating chemicals often act by unspecific cytotoxicity. It is likely that the same mechanisms that cause irritation are also responsible for the observed acute toxicity. The mechanism underlying the proposed acute toxicity QSAR could therefore be described as predominantly cytotoxic. One could argue, whether this definition would be a stringent enough mechanistic interpretation. So far, however, QSAR's based on mechanistic interpretations for modes of action of unspecifically reactive compounds are very restricted regarding their applicability domain. A pragmatic approach to define a mode of action, based on a simple *in vitro* test such as "irritation" may therefore be appropriate to increase structural diversity in the applicability domain.

The predicted classification of the compounds in the training and validation set showed that only 4% and 14% of the chemicals were underestimated regarding their acute oral toxicity. On the other hand, the developed QSAR had limited power to predict whether toxicity of a compound was overestimated by one class or not. Such an imbalance is not unique to this QSAR, but is inherent in each test where both, false positive and false negative outcomes are possible. The applicability of the proposed QSAR for acute toxicity is therefore best considered in the context of a tiered approach for chemical classification: a low probability of underestimation means, that for a compound with low toxicity additional *in vivo* testing would most probably not change the predicted classification. For compounds with a predicted high toxicity, however, chances are real that an *in vivo* test would change classification towards a lower acute toxicity class. This means that application of a QSAR, as proposed in this paper could result in the elimination of numerous unnecessary acute *in vivo* toxicity tests for non-toxic compounds and focus *in vivo* testing on cases with potentially higher risks for humans.



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## Structural Alerts for Aquatic Toxicity, Biodegradation and Bioaccumulation

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The OECD (Q)SAR Application Toolbox facilitates the formation of chemical categories and selection of chemical analogs in order to apply read-across, trend analysis or QSAR methods to fill data gaps. These approaches represent the realization of the concepts of integrated and/or intelligent testing strategies published recently and as outlined in the recent U.S. National Research Council report, *Toxicity Testing in the 21<sup>st</sup> Century*.

The U.S. EPA's Office of Pollution Prevention and Toxics (OPPT) and Office of Research and Development (ORD) have embraced the use of QSAR and expert system approaches in supporting regulatory decisions for industrial chemicals for the past 30 years. Several computerized QSAR and expert system tools have been developed by the U.S. EPA during this time and most of them rely on identification of structural features or "structural alerts" as an underlying organizing principle. For ecotoxicity, biodegradation and bioaccumulation, while the existence of multiple predictive models within U.S. EPA's predictive tools demonstrates predictive capability beyond the use of "structural alerts" as an initial profiling or grouping tool, the underlying alerts may still be useful for such purposes.

### ***Ecotoxicity***

ASTER, the Assessment Tools for the Evaluation of Risk, expert system assigns mode of action based on substructural "rules" and invokes a corresponding mode of action-based QSAR to provide toxic potency estimates. The ASTER knowledge base was derived from U.S. EPA's fathead minnow acute toxicity database and an international mode of action workshop held in 1988. The knowledge base was developed by expert examination of dose-response curves and behavioral responses, joint-action studies, Fish Acute Toxicity Syndrome (FATS) studies and published literature. As detailed in Russom et al., (1997), 461 chemicals were assigned to one of eight modes of action (including 'confidence score') and a validation exercise performed. The modes of action covered by ASTER include: narcosis I - baseline narcosis, narcosis II – polar narcosis, narcosis III – ester narcosis, oxidative phosphorylation uncoupling, respiratory inhibition, electrophile/proelectrophile reactivity, AChE inhibition, and CNS seizure response (covering several molecular mechanisms). The U.S. EPA would be amenable to including the fathead minnow database (800+ chemicals), the mode of action assignments and the sub-structure rules underlying ASTER mode of action assignments into the (Q)SAR Application Toolbox as structural alerts for profiling.

ECOSAR (version 0.99h) is currently included in the Phase 1 OECD QSAR Application Toolbox and the 44 ECOSAR chemical class assignments are available in the Profiling function of the Toolbox. This version of ECOSAR also includes 160 QSARs for the 44 classes based on confidential and publicly available experimental data. An update of ECOSAR (version 1.00) will be publicly released in the next few months. This version of ECOSAR will cover 130 chemical classes and include 440 QSARs, based on confidential and publicly available experimental data. Technical reference sheets (similar to a QMRF) will be available in the help menu and posted on-line for all chemical classes which will include the non-

confidential training set chemicals for each endpoint within a class. The “structural alerts” or definitions for each of the ECOSAR chemical classes and/or decision rules for assignment to a specific QSAR equation would be amenable to including into the (Q)SAR Application Toolbox as structural alerts for profiling. In addition, ECOSAR Version 1.00, including additional predictive models and the Technical Reference Sheets for each QSAR included in ECOSAR could be contributed to the Toolbox.

### ***Biodegradation***

U.S. EPA’s EPISuite includes 7 models, developed jointly by U.S. EPA and Syracuse Research Corporation, for predicting different types of biodegradation: BioWin 1 (Linear Biodegradation Probability), BioWin 2 (Non-Linear Biodegradation Probability), BioWin 3 (Survey Model – Ultimate Biodegradation), BioWin 4 (Survey Model – Primary Biodegradation), BioWin 5 (MITI - Linear Biodegradation Probability), BioWin 6 (MITI - Non-Linear Biodegradation Probability), and BioWin 7 (Anaerobic Linear Biodegradation Probability). Each of these models uses an approach in which molecular fragments are assigned a coefficient and the total probability of biodegradation is derived from summation of the coefficients and other factors (e.g., a molecular weight parameter is also incorporated for all but BioWin7). The methodology and validation exercises conducted in developing each of these models have been published in the peer reviewed literature (Howard et al., 1987; 1992; Boethling et al., 1989; 1994; Tunkel et al., 2000; Meylan et al., 2007). These publications and the EPISuite computer system contain the compiled empirical datasets as well as the expert-derived molecular fragments and the fragment ‘degradability’ coefficients associated with each. The structural fragments and coefficients may be amenable to including into the (Q)SAR Application Toolbox as structural alerts for profiling; however, using them individually as opposed to ‘summing’ them the way that the EPISuite program does is a limiting factor to be considered.

U.S. EPA also developed a biodegradation module as part of the ASTER expert system. This model was based on the peer reviewed publication by Niemi et al. (1987). Using 287 chemicals tested using standard BOD procedure, a “BOD half-life” (not an environmental degradation half-life) was derived and served as the fitted (dependent) variable. The training set consisted of 196 degradable ( $t_{1/2} < 5$  days) and 91 not degradable ( $t_{1/2} > 5$  days) chemicals. Twelve structural features or chemical classes were defined for degradable chemicals and 16 structural features or chemical classes were defined for non-degradable chemicals. This model is compared to other biodegradation models in a review by Howard (2000). The structural alerts derived by Niemi et al. are amenable for inclusion into the (Q)SAR Application Toolbox as structural alerts for profiling. Furthermore, if there is strong interest in incorporating this model into the (Q)SAR Application Toolbox, re-programming it into an amenable format may be pursued.

### ***Bioaccumulation***

Log  $K_{ow}$  has been the most common ‘indicator’ of bioaccumulation potential derived from structure. Based on the equilibrium partitioning approach, models describe the correlation between observed bioconcentration and the log  $K_{ow}$  of a chemical. This approach does not directly use information from chemical structure to make predictions (i.e., a fragment-based approach such as in models like KowWin or BioWin). Currently, there is a lot of research and development activity ongoing to refine/expand approaches for predicting bioaccumulation. Most are focusing on incorporation of pharmacokinetics, especially metabolism, into kinetic mass-balance models (e.g. Arnot and Gobas BAF/BCF model). These approaches are not a QSAR- or structural alerts- based per se. Rather, examples include: mechanistic approaches to incorporating structure and properties (e.g., Meylan et al. 1999 (BCFWIN), Dimitrov et al., 2002) and metabolism (e.g., Dimitrov et al., 2005); kinetic (mass-balance) approaches to predicting bioconcentration and bioaccumulation (e.g., Gobas 1993; Arnot & Gobas 2003) and incorporating ‘corrections’ using  $K_m$  (e.g. Arnot 2008). In addition, evidence is emerging regarding

the importance of considering air exposure/uptake and the use of  $K_{oa}$  as a predictive parameter, for terrestrial animals (Kelly et al. 2007).

In summary, the U.S. EPA, through development of predictive tools for aquatic toxicity (ASTER and ECOSAR) and biodegradation (ASTER and EPISuite), have identified a number of underlying structural fragments that would be amenable for inclusion in the OECD (Q)SAR Application Toolbox as “structural alerts” for use in the initial Profiling module to facilitate identification of chemical analogs and formation of chemical categories.

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## **Development of Metabolic Structural Alerts for Persistence, Bioaccumulation and Toxicity (PBT) Hazard Characterization**

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PBT profiling is a critical work flow component within the product life cycle of pharmaceuticals, agrochemicals and industrial chemicals. During the discovery and optimization phase, hazard characterization information is used in the design of molecules, not only with the desired chemistry and efficacy, but also with favorable PBT attributes. Our tiered testing strategy includes *in silico* profiling, lower tier (*in vitro*) high-throughput screening tests, followed by more targeted, higher tier testing to assess our molecules.

Estimation of the physical chemical properties, environmental behavior and toxicological potential using validated (Q)SAR and read-across methodologies are key components and accepted in the classification and regulation of chemicals by global regulatory authorities. One of the key technical challenges is to improve our current capability to estimate the chemical reactivity of an organic molecule in both the abiotic (hydrolytic, photolytic) and biotic compartments [soil, plants, non-target organism (fish), and mammals]. Several key issues need to be addressed are:

1. expand the knowledge based to include both open and proprietary information
2. cover diverse chemical structure features and biological properties
3. establish standardized searchable databases with data mining and knowledge extraction capability
4. assemble a reactivity knowledge base to describe both environmental and biological reactivity
5. develop an *in silico* toolbox and work flow for reactivity prediction
6. continue training and validation of the knowledge base

The scope of this presentation is to describe our approaches in the development of reactivity knowledge bases which include structural/substructural alerts. Our presentation includes:

1. building of a standardized electronic reaction databases, including both open and proprietary information
- 2, knowledge discovery work flow to build structural alerts that include hydrolysis, photolysis, soil (aerobic and anaerobic), plant, fish and mammalian metabolism

Several compound examples are presented to illustrate our work flow.

## Structural alerts and chemical reactivity in risk assessment

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Molecular Networks is performing contract work for the European Chemicals Bureau, Ispra, Italy on two software development projects:

ECB-1: Development of a rulebase for chemical reactivity, environmental and human fate and health effects

### START - SStructural Alerts for Reactivity in Toxtree

#### *Description of work*

- Compile a list of structural alerts for chemical reactivity
- Develop rules utilizing the structural alerts in logical decision trees
- Utilize results generated by QSARs
- Encode the rulebase as a software program serving as a plug-in to Toxtree

#### *Deliverables*

- A functional plug-in for Toxtree that allows categorization of chemicals into groups on the basis of structural alert

ECB-2: Development of a prototype software tool for predicting the fate of chemicals

### CRAFT - Chemical Reactivity And Fate Tool

#### *Aims of the project*

- Compile a list of structural alerts for chemical reactivity, including information on their domains of applicability
- Compile a list of QSARs for a range of chemical reactivity parameters
- Develop a rulebase capable of modelling reactions including the structural alerts
- Encode the rulebase in a software tool that allows for the prediction of reactive potential

#### *Requirements*

- The software tool must be flexible enough to incorporate additions to the knowledge base and rulebase
- The user interface must enable end-users to make these additions
- The software tool must have the capacity to interact with existing estimation tools (e.g. the OECD QSAR Application Toolbox, Toxtree, Ambit) and external databases

The major thrust of these projects is aimed at developing methodology for the modeling of chemical reactivity and the prediction of reaction products in the context of risk assessment of chemicals.

An understanding of chemical reactivity and the course and outcome of chemical reactions is required at several stages of the risk assessment process

- abiotic degradation of chemicals in the environment
- biodegradation
- metabolism in living species
- reactive toxicity

The concept of structural alerts can be transferred to the prediction of chemical reactivity only with caution. Clearly, for many decades the concept of functional groups – the equivalent of structural alerts – has served well for bringing order into organic chemistry in general and chemical reactions in particular.

However, any more detailed understanding of chemical reactions has to consider the mechanism of a reaction and the physicochemical effects influencing chemical reactivity. Approaches to a general treatment of chemical reactivity will be presented.

The implementation of START is building on Toxtree and that of CRAFT on Ambit, both open source software packages. Clearly, the development of open source in chemoinformatics is catching up. However, open source software also carries inherent problems such as long-term maintenance, adjustment to new requirements, on-time delivery of specific solutions, and requirements for new approaches.

Therefore, any software system in chemoinformatics that presents a stable, well maintained and state-of-the-art approach can not only rely on open source software but must also build on commercial software systems and databases. The implementation of START and of CRAFT in open source software will therefore also offer connections to proprietary software through web services.

*Molecular Networks GmbH, Erlangen, Germany ([www.molecular-networks.com](http://www.molecular-networks.com))* provides multifaceted, innovative software for drug design, combinatorial chemistry, planning of organic reactions and syntheses as well as for the prediction of physical, chemical, and biological properties of chemicals. The company commercializes about 15 software products that are used by more than 100 chemical and pharmaceutical companies worldwide. *Molecular Networks* has also successfully developed custom-designed applications for several industrial partners that resulted in long-term commercial relationships.

In the simulation of chemical reactions and the prediction of chemical reactivity, *Molecular Networks* can build on the expertise accumulated in the research group of Prof. Dr. Johann Gasteiger (the founder of Molecular Networks) of the Computer-Chemie-Centrum of the University of Erlangen-Nuremberg (<http://www2.chemie.uni-erlangen.de>) in the last three decades of research and development in chemoinformatics.<sup>1,2</sup>

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## Biotransformation Susceptibility of Chemicals in Aquatic Organisms - BiotS

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### Introduction

When aquatic organisms are exposed to chemicals in the environment, some of these chemicals maybe universally metabolised, whilst others are either metabolised by specific species/taxa or are non-metabolisable and therefore may bioaccumulate. The objective of this work was to develop a pragmatic approach to identify whether a query chemical maybe susceptible to biotransformation/metabolism in aquatic organisms and provide the user with data on the systems in which similar biotransformation reactions have been observed. The approach has been developed into a stand-alone named 'BiotS'.

### Methods

Data on the metabolic pathways of chemicals in aquatic organisms have been collated and analysed using regular expression pattern matching technology to identify structural fragments that are susceptible to biotransformation. These data were then encoded into a standalone software application called BiotS. The study dataset contained data on 85 biotransformation pathways involving 342 chemicals and 316 metabolic steps in 58 aquatic species.

### BiotS

BiotS was developed as a standalone desktop application with a graphical user interface (GUI) in the Sun™ programming the Java language version 1.5. The software allows the user to enter the structure of a query molecule into the software, using SMILES notation and then search for the presence of susceptible fragments. If susceptible fragments are identified the user can:

- Access the location of susceptible fragments to make a decision on their likelihood to biotransformation;
- Identify within which aquatic taxa the biotransformation has been observed;
- Access study information where the susceptible fragment has been observed biotransforming e.g. exposure duration and concentration; and
- Also access AMBIT descriptors, metabolic pathway(s) and bioproperty data for chemicals where the susceptible fragments have been reported to be metabolised in the publicly available literature.

### Future Work

Recently a follow-on project has been secured to develop the BiotS software to include data on biotransformation observed in other taxa e.g. mammals and microbes as well as investigate fragments that are not susceptible to biotransformation.

### Acknowledgements

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## **A Decision Support System for Prioritizing ER Binding Potential Within Defined Regulatory Inventories: A Chemical Sub-class Approach**

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### **PLEASE NOTE:**

*The research being conducted on in vitro and QSAR methods was not sufficiently developed to be ready for use in the initial round of USEPA EDSP priority setting, but may be used in subsequent rounds of EDSP priority setting (<http://www.epa.gov/scipoly/ospendo/prioritysetting/approach.htm>). Mention of a substance in the meeting summary or EPA presentations concerning the development of such methods does not mean the Agency has or will make a determination that any use of the substance will pose a significant risk. Further, this research is insufficient to allow EPA to draw any conclusions as to whether these substances are "endocrine disruptors"; the substances listed are simply compounds that have been or may prove to be useful in developing ranking and prioritization methods.*

### **Background:**

A challenge facing the US EPA Office of Pesticide Programs (OPP) in implementing the Food Quality Protection Act is a need to prioritize testing for classes of compounds where 'endocrine data' is lacking. This specifically applies to inert ingredients used in the formulation of pesticides used on food crops, and antimicrobial active ingredients. A QSAR-based Decision Support System for chemical prioritization was developed within the context of this defined risk assessment problem (e.g., prioritization of these defined inventories of concern for further testing), and for a well-defined adverse outcome pathway. The approach allows assessment of what chemical structures are most likely to initiate the pathway under study by systematically varying structure and measuring resultant activity, using *in vitro* assays focused on initiating events within an adverse outcome pathway. While specific measures are made in *in vitro* assays, the adverse outcome pathway concept is employed to articulate the linkages between initiating events and ultimate *in vivo* adverse outcomes which are the focus of risk assessments. The pathway articulation may include description of observations made at various levels of biological organization, and using *in vitro* or *in vivo* systems. Thus decisions on the type and extent of further testing is made with consideration of the what is or is not known regarding the toxicological consequence of specific chemicals and their interactions with biological systems.

### **A Systematic Approach to Developing a Decision Support System:**

A multi-faceted approach was used to determine the chemical structural requirements for initiation of a distinct toxicity pathway linked to an adverse outcome of regulatory concern. Many aspects of the systematic approach have been reported previously including: an overview of the conceptual approach (Schmieder et al., 2003); optimization of assays for the applications under study (Schmieder et al., 2000); comparing effects among species (Denny et al., 2005); and demonstrating the use of *in vitro* assays along a plausible estrogen receptor (ER)-mediated toxicity pathway (Schmieder et al., 2004). Use of the assays for systematic testing to cover the chemical classes found on the OPP lists resulted in the development of a database that is specifically applicable to the chemical structures of interest. Understanding the regulatory need, and for what chemicals and which endpoint, are key steps in a developing a robust quantitative structure-activity relationship (QSAR) approach. Briefly, the approach incorporated: a) defining the regulatory question (i.e., prioritization chemicals for further testing for potential estrogenicity); b) identifying the regulatory target, (i.e., specifically defining the chemicals in question and characterizing the

discrete compounds on the lists obtained); c) defining the adverse outcome of regulatory concern (ER-mediated toxicity pathway leading to reproductive impairment); d) for key events along the pathway, optimization of *in vitro* assays for the types of chemicals found on the regulatory lists; e) use of QSAR-based hypothesis generation and strategic chemical selection for testing; f) targeted testing within chemical classes/sub-classes; g) collecting the derived information in a Decision Support System incorporating mechanistic interpretation where possible; and, h) using the Decision Support System to prioritize untested chemicals.

The iterative nature of the process allowed new knowledge gained to be added to the QSAR-based system, and mechanistic understanding to grow as the work progressed. This new knowledge was used to refine hypotheses and further target the testing. It became apparent early on that the chemicals on EPA lists in question were not likely to be the type of high potency chemicals there were the primary focus of early studies on chemical interactions with the ER. Thus, early focus was on obtaining a better understanding of how low affinity chemicals interact with the ER, including ensuring that the assays used were optimized to detect an effect due to these types of chemicals. This required careful attention to influence of chemical physical-chemical properties on the interactions of chemicals with the abiotic and biotic components in the assay systems, as these properties varied widely for the chemicals on the target inventories.

The approach used is grounded in seeking a mechanistic understanding of underlying chemical-biological interactions within a specific adverse outcome pathway; and grouping chemicals within a mechanistic class based on similar biological activity. To that end, subsets of chemicals were tested for two primary purposes: i) to verify mechanistic hypotheses of chemical interaction with the ER for low affinity compounds; and ii) to gain knowledge specific to chemical classes and sub-classes found on the EPA lists. Chemical classification tools were developed for grouping, comparing, and evaluating chemicals on OPP lists with chemicals structure that have undergone testing. The tools facilitate identification of subgroups of chemicals for testing and evaluation to maximize information gained from each newly tested structure. Additional chemicals were selected for testing, especially to evaluate acyclic chemicals which were a large percentage of OPP inventories.

### **Results and Conclusions:**

The categorization in the Decision Support System by chemical classes/sub-classes was automated as a series of sub-structural queries (e.g., structural alerts) and was used to prioritize the discrete chemicals on the OPP lists. The result was that >90% of the chemicals belonging to the classes/sub-classes on the lists have a low potential to bind ER and initiate ER-mediated gene expression, based on tested members of the class/sub-class, or on other criteria (e.g., class-specific Log Kow cut-offs). Thus the approach allows the identification of negative sub-classes in the context of the activity being measured and the definition of a sub-class specific to the chemical lists under study. Caution must be taken not to inappropriately apply the information to sub-classes of chemicals outside the defined ranges. The chemicals sub-classes that have a higher potential to bind ER are provided along with a mechanistic interpretation of their interaction with the receptor.

The approach described that resulted in the development of a Decision Support System incorporates OECD QSAR principles, where the regulatory question is well-defined, the assays are used in the context of a defined toxicity pathway leading to the adverse outcome of regulatory concern, the assays were optimized to be applicable to the endpoint and types of chemicals under study, the sorting of chemicals in classes/sub-classes and rationale for decisions are transparently presented in the Decision Support System, and mechanistic interpretation is provided.

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## Structural Alerts for Protein Binding: Sub-categories & Adding the Dimension of Potency

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The OECD QSAR Application Toolbox (hereafter Toolbox) applies computational methods to filling data gaps by facilitating the selection of chemical analogues and grouping chemicals into categories. It integrates (among other things) mechanisms of action with categories. The Toolbox contains 17 different profilers or grouping methods, which vary in design, specificity, and completeness. Among the best characterized profilers is the one for protein-binding, which integrates mechanisms of action with categories. This profiler is important to excess toxicity for aquatic organisms, acute toxicity via inhalation of gas or vapour, sensitization, both skin and respiratory, and chromosomal aberrations. Weak (slow reacting), direct-acting electrophiles and pro-electrophiles (compounds that are metabolized to electrophiles; typically Michael acceptors) also have the potential to cause irreversible effects associated with developmental toxicity and specific target-organ toxicity, including liver and kidney.

The Toolbox's protein-binding profiler categorizes 38 different mechanisms. The mechanisms vary in specificity and completeness. Among the best characterized are the ones for S<sub>N</sub>2 nucleophilic addition. Among the poorer characterized are the ones for Michael type nucleophilic addition and S<sub>N</sub>Ar nucleophilic addition.

In the analogue approach endpoint information for a single or small number of tested chemicals is used to predict the same endpoint for an untested chemical that is considered to be "similar". The analogue sets of chemicals are often selected based on the hypothesis that the toxicological effects of each member of the set will show a common behavior or consistent trend. The advantage of the analogue approach is that identification of consistent patterns of a measured effect within an analogous group increases confidence in the reliability of the results for individual compounds within the analogous series, which in turn increase confidence in the prediction.

In read-across the presence or absence of an activity for the untested chemical of interest can be inferred from the presence or absence of the same activity for the tested analogue(s). In qualitative read-across, the analogous series can be defined solely by the mechanism of action (e.g., Michael type nucleophilic addition) with the applicability domain being simply defined (e.g., polarized a,b-unsaturated) (Enoch et al., 2008).

In quantitative read-across a potency value for tested analogue(s) is used to estimate the potency for the untested chemical with the assumption that the potency of the effect of interest is shared by both the tested and untested analogue. Meeting the assumption of shared potency may require the use of sub-categories within a given mechanistic category. Such sub-categorization, while often endpoint specific, improve practicality, flexibility, and predictivity. Importantly, sub-categorization does not alter scientific-basis of the category approach.

Examination of *in vivo* toxicity data typically reveals incomplete representation of protein-binding mechanisms, skewed representation of the applicability domain of those mechanism that are present and incomplete representations of the sub-domains of many protein-binding mechanisms. Therefore, derivation of numerical values for quantitative read across may be fraught with uncertainty.

*In Chemico* reactivity quantified in a rapid, inexpensive method based on model nucleophiles is demonstrated to be a means of verify mechanism-based rules of reactivity, define the applicability domain of a reactive mechanism and provides a measure of relative potency (Schultz et al., 2006).

The protocol we use (Schultz et al., 2005) is a spectrophotometric-based depletion method; measures % free thiol with GSH as the model nucleophile. The endpoint,  $RC_{50}$  (50% reactive concentration; mM) calculated by probit analysis of concentrations-response data after 2-hrs is linearly related to rate constants. Data derived from this experimental approach has used to form subcategories for potency based read-across (Schultz et al., 2007).

An application of reactivity potency is demonstrated for cinnamic compounds, which are associated with fragrance. Cinnamic compounds are based on vinyl benzene (c1ccccc1C=C) with substituted of the  $\beta$ -carbon of the vinyl group. There are 35 cinnamic derivatives in the 1500 substances reported on the "List of Fragrance Chemicals". Twenty-two of these cinnamic derivatives are esters, 7 are aldehydes, and the remainder alcohols or substituted with other polarized groups. Categories and subcategories of the fragrance-related cinnamic compounds reveals 14 classified as non-protein binders, 3 classified as pro-Michael acceptors, and 18 classified as Michael acceptors. Of the latter group 2 fast reacting vinylene aldehydes, 12 moderately reacting alkyl cinnamates, and 3 slow reacting methacrylate-like cinnamics or cinnamyl nitrile.

Chemical reactivity data can easily be programmed using SMARTS to identify potency-related subcategories (Schultz et al., 2007). Adding structural alerts for sub-categories to selected protein-binding categories in the Toolbox has the potential of improving predictability by the read-across and trend analysis methods.

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## **Alert-based Toxicity Prediction at Lhasa Limited**

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In the late 1970's and early 1980's a team led by Professor E. J. Corey at Harvard University developed a knowledge-based expert system for chemical synthesis planning, "LHASA". The program showed potential for use in commercial research but it needed further development. By then, a unit at Leeds University under Dr A. P. Johnson was working on the project in partnership with the one at Harvard, and group of companies in the UK agreed to sponsor the programming research and to do the knowledge base development. A not-for-profit collaboration was set up initially as LHASA UK Limited, which became Lhasa Limited.

Early sponsors of the project had full source code for the program, and staff at Chesterford Park Research Station used the chemical perception capabilities of LHASA to create a prototype alert-based system for predicting toxicity, DEREK. Schering Agrochemicals Limited, then owners of Chesterford Park Research Station, donated DEREK to Harvard and Lhasa Limited, where it was developed into a working system.

Alert-based prediction is useful but it does not take account of the effects of physical properties and it does not deal satisfactorily with cases where activity against an end-point is actually known for the query. Many assays for predicting toxicity do not measure the end-point itself. The Ames test may indicate potential carcinogenicity but it does not measure carcinogenicity. Ideally, an expert system should report an alert for Ames test activity as just that. Instead of predicting carcinogenicity directly from the alert, the system should contain separate rules about activity-activity relationships. So DEREK was superseded by Derek for Windows, which makes predictions by chaining together sets of rules.

Lhasa Limited wants to support the OECD Toolbox project. Under charity regulations we must make best use of our assets. Allowing appropriate organisations with limited funds to use Lhasa software free of charge would be considered a proper use of our assets: releasing the software for uncontrolled use free of charge by organisations currently willing to pay for it would not be. So what can Lhasa contribute to the Toolbox?

The alerts that went into the original DEREK could be donated to the OECD project for free public access because they have now been fully depreciated. A few more alerts that have been described in publications from Lhasa Limited could also be donated. There would be some technical difficulties with transferring the alerts to the Toolbox from the original DEREK and from Derek for Windows but they are not insurmountable. However, there is a concern. The OECD Toolbox would contain a subset of alerts from Lhasa Limited and no higher-level rules. If this is to be done, ways must be found to ensure that users understand the limitations and find them acceptable. Otherwise the images of both Lhasa Limited and the OECD may be damaged.

Lhasa Limited could work on collecting new data and/or deriving alerts from it for inclusion in the Toolbox. If the work could be funded at cost the data and alerts would be made public free of further charge. Lhasa would want to be allowed to include the data and alerts in its own software as well (this would also be free of charge unless the OECD thought it should be otherwise).

Finally, Lhasa would be very happy to do work on development of the Toolbox itself. What work needs to be done, and how can we help with it?

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